INVESTIGATION OF INTERACTIONS BETWEEN THE 193-NM ARGON-FLUORIDE EXCIMER LASER AND CORNEAL TISSUE

By

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by

Brian T. Fisher
This work is dedicated to my wife and best friend Dawn, whose support and encouragement made its completion possible.
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INVESTIGATION OF INTERACTIONS BETWEEN THE 193-NM ARGON-
FLUORIDE EXCIMER LASER AND CORNEAL TISSUE

By

Brian T. Fisher

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Chair:  David Hahn
Major Department:  Mechanical and Aerospace Engineering

Laser corneal refractive procedures, including photorefractive keratectomy (PRK)
and laser-assisted in situ keratomileusis (LASIK), have become immensely popular for
the correction of imperfect visual conditions such as myopia and astigmatism. These
procedures involve the distribution of carefully controlled laser pulses over the treatment
zone to reshape the corneal surface, which alters the refractive power and shifts the focal
point of the cornea. The clinical laser systems are technologically advanced and very
precise in many ways, allowing removal of corneal tissue with submicron accuracy.
However, these systems rely on empirically determined ablation rates of corneal tissue,
sometimes leading to unexpected and unacceptable surgical outcomes.

The ablation process itself, including the fundamentals of the interaction between
the laser light and the corneal tissue as well as the mechanics of the actual tissue removal,
is not well understood. Therefore, it has not been possible to measure accurately the
ablation rate of corneal tissue and determine its relationship to variable parameters such
as corneal hydration and laser fluence. A practical goal of the present research is to
determine an easily implemented method for measuring *in situ* the ablation rate, thereby
providing feedback in clinical systems. More fundamentally, however, the focus is on a
better understanding of the mechanisms, whether photochemical or thermal, of the
ablation process itself. A better fundamental understanding of the ablation process is
expected to aid in the formation of a comprehensive model, which may then be used to
predict tissue ablation rates.
CHAPTER 1
INTRODUCTION

1.1 Anatomy and Physiology of the Cornea

1.1.1 Overview of the Cornea and the Eye

The outer surface, or outer tunic, of the eye consists of the transparent cornea and the opaque sclera. The cornea covers one-sixth of the surface and the sclera covers five-sixths, and the two sections meet at the corneoscleral limbus. The cornea is circular at the inner (posterior) surface and oval at the outer (anterior) surface, with a slightly larger diameter in the horizontal plane than in the vertical. The central one-third of the anterior surface is called the optical zone and it is nearly spherical, and the anterior surface as a whole provides the majority of the eye’s refractive power. Because the posterior surface of the cornea is more spherical than the anterior, the peripheral cornea is typically about 650 to 670 µm while the central cornea is typically about 500 to 570 µm in thickness (Klyce & Beuerman, 1998; Lerman, 1980). Studies have shown that corneal thickness may vary between ethnic groups as well as with age (Faragher, Mulholland, Tuft, Sandeman & Khaw, 1997; Hahn, Azen, Ying-Lai & Varma, 2003; La Rosa, Gross & Orengo-Nania, 2001).

Behind the cornea is the anterior chamber of the eye, which contains the iris and the lens. The lens provides some refraction and helps to focus light to create images on the retina. Surrounding the lens is the iris, which is a muscular structure that tenses or relaxes to control the amount of light passing through the lens. The opening in the center of the iris is known as the pupil, and it allows more light into the eye when it is dilated.
and less light into the eye when it is constricted. The aqueous humor is the clear fluid that fills the inside of the anterior chamber and provides nutrients to the lens, iris, and cornea. Behind the lens and filling the center of the eye is the vitreous humor (or vitreous body), which is a gelatinous substance that gives the eye its overall shape and form. The retina is the innervated area on the posterior surface of the eye onto which light is focused to create an image. A basic anatomical diagram of the eye, adapted from the Lion’s Eye Health Program Web site (2003), is shown below in Figure 1-1.

![Figure 1-1. Anatomy of the eye.](image)

1.1.2 Layers of the Cornea

The cornea is essentially avascular, and therefore it obtains oxygen from the adjacent atmosphere through the anterior surface and it obtains nutrients from the interior of the eye through the posterior surface. There are five major layers that constitute the cornea. From anterior to posterior, the layers are the epithelium, Bowman’s layer, the stroma, Descemet’s membrane, and the endothelium. A cross-section of a typical cornea, adapted from the Webb Microtome Web site (2002), is shown in Figure 1-2.
There is a thin tear film that lines the outer surface of the epithelium, but this film is not truly a part of the cornea. The epithelium includes up to six layers of cells and constitutes approximately 10% of the total corneal thickness. At the innermost part of the epithelium lies the basal cell layer, which is responsible for creating new epithelial cells almost constantly. Below the epithelium is Bowman’s layer or Bowman’s membrane, which itself consists of two layers. The anterior portion is the basement membrane of the basal epithelium, and the posterior portion is a somewhat amorphous section consisting of bundles of collagen fibrils. It is commonly accepted that Bowman’s layer cannot regenerate when injured, unlike the epithelium. It is also commonly accepted that Bowman’s layer is prominent in primates, while other mammals have only a very thin zone that is suggestive of a Bowman’s layer (Klyce & Beuerman, 1998).

The central layer of the cornea, the stroma, consists of stacks of collagen fibrils, called lamellae, and constitutes nearly 90% of the total corneal thickness (Klyce &
Beuerman, 1998). With a thickness of about 20-25 nm, the bundles of collagen fibrils in the stroma are about one and a half times as thick as the bundles in Bowman’s layer (Klyce & Beuerman, 1998; Lerman, 1980). Cell density in the stroma is very low, with the collagen bundles separated by approximately 60 nm (Lerman, 1980). Keratocytes, which function to maintain the stromal constituents (fibrils and extracellular matrix) by constant synthesis, occupy a small percentage of the stroma and typically lie between collagen lamellae. As a whole, the stroma consists of 78% water, 15% collagen, and the remaining 7% other materials (Siew & Clover, 1995). Furthermore, collagen represents 71% of the dry weight of the cornea and, despite the fact the cornea is relatively thin, it has remarkable inherent strength owing largely to the collagen in the stroma (Klyce & Beuerman, 1998).

The remaining two layers, Descemet’s membrane and the endothelium, are closely related. The endothelium is a single layer of flat cells with a very smooth posterior surface. Descemet’s membrane is a thick basal lamina that is secreted by the endothelium, and it is loosely attached to the posterior of the stroma.

1.1.3 Collagen Structure

To date, no published work has thoroughly described the structure of collagen, the primary constituent after water in corneal tissue, as it relates to photochemistry. Some presentations include discussions of basic collagen structure, but these treatments are generally cursory (Kitai, Popkov, Semchishen & Kharizov, 1991).

For clarity and simplicity, the overall structural unit of collagen will be referred to as the macromolecule. The collagen macromolecule is a right-handed, triple helix structure, where each helical strand is composed of a series of amino acids. The strands are linked together by bonds known as covalent cross-links, which provide structural
integrity. The sequence of amino acids in a particular strand is a quasi-repeating pattern, typically represented as Gly-X-Y, where glycine (Gly) appears as every third residue, X is generally considered proline, and Y is generally considered hydroxyproline. Other amino acids may appear in trace amounts in place of the proline and hydroxyproline, but collagen is most conveniently modeled as a repeating sequence of glycine, proline, and hydroxyproline in overall equal proportions. The chemical formulas for glycine, proline and hydroxyproline are \( C_2H_5NO_2 \), \( C_5H_9NO_2 \) and \( C_5H_9NO_3 \), respectively. The three amino acids are linked to form the repeating sequence found in collagen, as depicted in Figure 1-3 (Lerman, 1980; Murray & Keeley, 2003; Nelson & Cox, 2000a, 2000b; Rodwell & Kennelly, 2003; Schultz & Liebman, 2002; Zubay, 1998).

![Figure 1-3](attachment:image.png)

**Figure 1-3.** Repeating sequence forming the primary structure of collagen. The thick lines between each amino acid represent peptide bonds.

The collective chemical formula for this repeating sequence of three amino acids is \( C_{12}H_{17}N_3O_4 \), which sums to 267 atomic mass units (amu). Note that the peptide bonds are formed through a substituted amide linkage, which also forms a water molecule (i.e., dehydration); hence the total atomic mass is different than the sum of the respective amino acids. The average weight of a typical collagen macromolecule is approximately
308,300 amu, which corresponds to 1155 groups of the repeating amino acid sequence. It is generally accepted that the primary chromophore for 193-nm excimer laser light in corneal tissue is the peptide bond (C-N) between adjoining amino acids (Coohill, 2002; Wetlaufer, 1962). Accordingly, one may calculate a total of 3465 chromophores for 193-nm radiation present in each macromolecule of collagen, based on three peptide bonds per repeating amino acid sequence (i.e., glycine-proline-hydroxyproline) and 1155 sequences per macromolecule. Quantification of the absorption characteristics of the purported peptide bond chromophore is one of the goals of this doctoral research.

1.2 Refractive Conditions

Two-thirds of the focusing power of the eye resides in the cornea, and this power is fixed. The remaining one-third is due to the lens, and this power is flexible due to a process called accommodation (Bower, Weichel & Kim, 2001). Refractive power is the reciprocal of focal length, such that a refractive power of two diopters corresponds to a focal length of 0.5 m. Perfect refraction is called emmetropia. In this case, the light rays from a distant object focus directly onto the surface of the retina and the object appears normal. Myopia is a condition in which the eye’s power is too strong and light rays from a distant object focus at a point in front of the retina. This condition, the most common refractive error, is commonly referred to as “near-sightedness” (Bower et al., 2001). Hyperopia is a condition in which the eye’s power is too weak and light rays from a distant object focus at a point behind the retina. This condition is more commonly known as “far-sightedness.” Astigmatism, which can exist simultaneously with emmetropia, myopia, or hyperopia, is a condition in which the anterior surface of the cornea is more elliptical than spherical, and therefore light rays from a distant object actually focus at two different points (Manche, Carr, Haw & Hersh, 1998).
1.3 Corneal Refractive Surgery

Many types of refractive surgeries have been developed to reshape the surface of the cornea, the earliest of which did not involve any type of laser technology.

1.3.1 Non-Laser Procedures

Until the mid-1990’s, radial keratotomy (RK) was a popular refractive surgery for patients suffering from myopia. In this procedure, a surgeon makes microscopic radial incisions in the cornea in a “spoke-like pattern” (Bower et al., 2001). The incisions allow the peripheral cornea to relax which results in flattening of the central cornea and refractive correction. The new shape is theoretically permanent once the cornea heals.

Some major concerns associated with this procedure are perforation of the cornea, infection, and rupture of the eye globe. Also, the lack of precision associated with RK can lead to overcompensation, meaning a shift from myopia to hyperopia. Radial keratotomy has become nearly non-existent since the U.S. Food and Drug Administration (FDA) approved the use of excimer lasers for corneal refractive procedures in 1995. In 2001, a survey was conducted of 980 U.S. members of the International Society of Refractive Surgery (ISRS). It was reported that the percentage of ISRS U.S. refractive surgeons that perform RK had declined over the previous five years from 20% to less than 1% (Duffey & Leaming, 2002).

1.3.2 Excimer Laser Procedures

Early studies found that the ArF excimer laser, operating at a wavelength of 193 nm, can be used to precisely shape the cornea because of the strong absorption of far-UV light by corneal tissue. It was suggested that the laser light could be applied as a broad beam, using a circular mask to distribute the light more centrally or peripherally, depending on the desired result (Srinivasan & Braren, 1989; Waring, 1989). The idea of
Excimer laser refractive surgery was that the laser would precisely perform the surgical procedure in an essentially automated manner, and the surgeon’s expertise would be used for patient selection, diagnosis, proper alignment of the laser before and during the procedure, and drug prescription. Despite the precision of the laser and delivery system, however, the corneal wound healing response is somewhat unpredictable and leads to imprecision in the refractive result (Waring, 1992).

Photorefractive keratectomy (PRK) was the earliest laser refractive surgical procedure. In this procedure, the corneal epithelium is removed by one of a variety of methods, including manual scraping, a rotating brush, chemical removal, or by the excimer laser itself (Manche et al., 1998; Odrich & Greenberg, 1998). The exposed corneal surface is then ablated with the excimer laser in a prescribed pattern to remove tissue from specific locations and reshape the cornea. Perhaps the most important issue during a PRK procedure is centration of the ablation pattern over the pupil, which is maintained by having the patient stare at a fixed target. Decentrated ablation is reportedly the most common error committed by inexperienced surgeons (Odrich & Greenberg, 1998).

Other complications include undercorrection, overcorrection, refractive regression, the formation of central islands, and corneal haze. Overcorrection can have a frustrating and debilitating effect, because patients who were myopic and were able to focus on objects by bringing them closer may need to move them farther away to see them clearly after the PRK procedure. Central islands are highly localized areas of relative central corneal steepening, and they may result in glare, halos, or other visual problems (Odrich
Greenberg, 1998). Central islands and corneal haze are generally transient issues that diminish postoperatively within a few months.

Laser-assisted *in situ* keratomileusis (LASIK) was developed using the same laser technology as PRK. A microscopic knife blade, called a microkeratome, is used to create a lamellar flap, including the epithelium and Bowman’s layer, which remains hinged to the eye. The flap is pulled back to expose the corneal stroma, which is then ablated with the excimer laser. When the ablation is complete, the flap is repositioned and can be left alone, sutured, or covered with a protective contact lens (Buratto & Ferrari, 1992; Pallikaris, Papatzanaki, Siganos & Tsilimbaris, 1991). The use of a protective contact lens is the most common methodology. Even the earliest studies suggested that leaving the anterior cornea, including Bowman’s layer, intact and directly ablating the stroma may allow quicker healing and a more predictable result (Pallikaris, Papatzanaki, Stathi, Frenschock & Georgiadis, 1990).

Complications related to LASIK, similar to PRK, include decentration, undercorrection, overcorrection, refractive regression, central islands, and postoperative haze. However, LASIK may involve other complications, mainly related to the flap. These complications include incomplete or irregular flaps, free caps, flap wrinkles, and infection (Farah, Azar, Gurdal & Wong, 1998; Slade, Machat & Doane, 1998). In particular, inflammation at the flap interface during healing, known as diffuse lamellar keratitis, is not uncommon. In addition, it has been suggested that variation in how LASIK is performed, including how the flap is created and how the eye is protected after the procedure, leads to non-ideal outcomes (Rosen, 2001).
There have been many advances since the advent of LASIK that have changed and generally improved the procedure. Alcon, a leading manufacturer of clinical excimer laser systems, has incorporated an active eye tracking system called LADARVision that alters the laser beam path to compensate for eye movements (Krueger, 1999). This technology allows the surgeon to use the scanning laser to smooth out small surface irregularities. Wavefront sensing is a commonly used method of mapping the surface of the eye to more accurately determine the ablation pattern (Schwiegerling, 2002). A low-power array of light beams is sent into the eye and focused onto the retina, which then scatters light in spherical wave fronts that are altered as they pass back through the eye. A detector array collects the scattered light and creates an image of the aberrations of the eye. The most common analysis scheme uses the Shack-Hartmann algorithm to interpret the wavefront data and predict the needed change in refractive power to correct all refractive errors, including myopia, astigmatism, and higher order aberrations (Davies, Diaz-Santana & Lara-Saucedo, 2003; Platt & Shack, 2001).

Very recently, a new way of creating the corneal flap using a femtosecond laser has been suggested (Juhasz et al., 2002). The femtosecond laser “blade” may offer the refractive surgeon greater control over parameters such as the flap thickness, diameter, and hinge position. Also, a procedure known as LASEK (laser epithelial keratomileusis) has been developed as an alternative to LASIK (Camellin, 2003). In LASEK, an alcohol solution is applied to loosen the edges of the epithelium, allowing the surgeon to create an epithelial flap and ablate relatively superficial tissue. The potential advantages of LASEK are the elimination of stromal flap complications and the ability to perform corrective laser refractive surgery on patients with relatively thin corneas.
LASIK has become the standard corneal refractive procedure preferred by most ophthalmologists and ophthalmic surgeons, as indicated in a 2001 survey of the ISRS (Duffey & Leaming, 2002). This survey indicated that over the five years prior to 2001, ISRS U.S. surgeons performing PRK decreased from 26% to less than 1%, while LASIK performance increased dramatically from 39% to 85%. A similar survey of refractive surgeons the following year reiterated the preference of LASIK over other surgical methods (Duffey & Leaming, 2003). As discussed above, notwithstanding the potential surgical complications, LASIK is the preferred procedure because it generally allows quicker healing and has a more predictable outcome.

Statistics show that laser refractive surgery continues to soar in popularity. In 2001, 1.3 million procedures were performed in the United States, with LASIK accounting for over 92% of these (Harmon, 2001, 2002). In 2003, over 1.5 million people underwent laser refractive surgery (Fischetti, 2004). The long-term stability and effectiveness of laser refractive surgery remain unknown, as ophthalmologists have data spanning only a decade. Clearly, however, the general success of these procedures in the short-term has proven sufficient to attract new patients.

1.4 Clinical Outcomes

1.4.1 PRK Results

Gartry, Kerr Muir and Marshall (1991) performed a detailed clinical study, using 120 sighted eyes, to assess the safety of PRK as well as the predictability and stability of the refractive outcome. After one week postoperatively, the average patient experienced an overcorrection of his or her myopia, and the magnitude of this overcorrection was proportional to the magnitude of the intended correction. The mean refractive result after twelve weeks was very close to the desired result, but with a great deal of individual
variation. Over 90% of the patients experienced postoperative corneal haze, and younger patients experienced a greater amount of haze than older patients. Complications and side effects included a foreign body sensation (18% of patients), decentration (1 patient), a noticeable rise in intraocular pressure (12%), a halo effect at night (78%), and various levels of discomfort (20%). Most of the side effects decreased over time, and the decentration case occurred because the patient moved significantly during the procedure. This study found PRK to be safe and effective overall, but more importantly found that the procedural accuracy decreased as the attempted correction increased. The authors offered three possible reasons—an error in the treatment algorithm that accumulates for more laser pulses, an increased tissue healing response as the ablation grows deeper, or a gradient in the ablation rate through the stroma.

Hersh, Schein and Steinert (1996) performed a similar study on one eye of each of 701 patients to determine the safety and effectiveness of PRK for the treatment of myopia. As with the above study, the procedure performed in this study involved the use of a microsurgical blade to remove the epithelium. Two years postoperatively, 78% of the patients had a refractive change that was within one diopter of the intended correction. It was also found in this study, just as in the above study, that larger intended corrections had a reduced success rate. The authors suggested that corneal hydration may have affected the laser-tissue interaction, theorizing that shockwaves associated with each laser pulse may drive water into the ablation zone and lead to a decreased ablation rate.

Vetrugno, Maino, Valenzano and Cardia (2001) investigated the temperature of the cornea before and after PRK using non-contact infrared (IR) thermometry. The PRK procedure, including the use of a blade to remove the epithelium, was performed on 58
patients using a scanning laser rather than a broadbeam laser. Even at a pulse repetition rate of 100 Hz, it was found that the temperature of the cornea never increased by more than about one degree Celsius. This led to the conclusion that thermal effects during laser refractive surgery are negligible.

1.4.2 LASIK Results

There is considerable literature reporting the results of LASIK for the treatment of myopia, hyperopia, and astigmatism (Barker, Couper & Taylor, 1999; Chayet, Magallanes, Montes, Chavez & Rabledo, 1998; Gibralter & Trokel, 1994; Jackson, Casson, Hodge, Mintsoulis & Agapitos, 1998; Lavery, 1998; Ojeimi & Waked, 1997; Pallikaris & Siganos, 1997; Perez-Santonja, Bellot, Claramonte, Ismail & Alio, 1997; Wang, Chen & Yang, 1997). Much of the interest in LASIK developed because surgeons wanted to find a treatment for high myopia, since PRK was found to be ineffective for correction of more than six diopters of myopia. Mixed results have been achieved, with Pallikaris and Siganos (1997) reporting that LASIK is effective for the treatment of myopia greater than six diopters, while Perez-Santonja et al. (1997) reported that LASIK for high myopia resulted in poor predictability, high regression, and relatively severe corneal haze. Wang et al. (1997) found that LASIK for low to moderate myopia resulted in relatively quick healing, little to no corneal haze, and a good refractive result, and also suggested that LASIK may be promising for high myopia. Barker et al. (1999), however, discovered that epithelial hyperplasia, or postoperative thickening, might cause significant refractive regression after LASIK.

Ojeimi and Waked (1997) and Jackson et al. (1998) found LASIK to be an effective treatment for hyperopia, as long as it was used to correct only low hyperopia. Other studies have shown that LASIK is reasonably effective for the correction of
hyperopia and hyperopia combined with astigmatism, while also concluding that procedural predictability and long-term stability remain unproven (Ibrahim, 1998; Suarez, Torres & Duplessie, 1996). It was found in another study that LASIK is successful in treating eyes that have been overcorrected for myopia and have therefore become hyperopic (Jacobs et al., 2001). The same study also concluded that it made little or no difference whether the original LASIK flap was lifted or whether a new flap was cut. Chayet et al. (1998) and Gibralter and Trokel (1994) found LASIK to be an effective procedure to smooth out an astigmatic corneal surface.

LASIK retreatment studies were performed by Durrie and Aziz (1999) and Lyle and Jin (2000) with relative success. Durrie and Aziz (1999) retreated twelve eyes that were undercorrected for myopia by lifting the already existing flap and performing laser ablation within one to nine months after the original treatment. They found the retreatment procedure to be successful and free of complications, but also warned that retreatment should only be done if it is certain that the undercorrection is stable and that the refractive power of the eye is not still in a transient phase. Similarly, Lyle and Jin (2000) found retreatment to be safe and effective. They even suggested a procedure for correcting high myopia that includes an initial treatment with an intended undercorrection followed by a retreatment to correct the residual myopia. Vorotnikova, Kourenkov and Polunin (1998) performed PRK retreatment on myopic eyes that showed relatively significant regression at least six months after the initial PRK treatment. The preliminary results were satisfactory and stable at six months after retreatment.

Two important technologies that have been developed recently are eye tracking systems and wavefront-sensing. Taylor, Eikelboom, Van Saarloos and Reid (2000)
reported that the inclusion of an eye tracking system in laser systems for refractive surgeries led to better alignment of the laser with the eye and therefore decreased the likelihood of decentration during ablation. Clinical studies on human patients by Mrochen, Kaemmerer and Seiler (2000, 2001) have shown evidence that wavefront-guided LASIK procedures lead to very good refractive results. In the 2001 study, it was found that at three months postoperatively, 68% of the eyes were within 0.5 diopters of emmetropia and 93.5% were within one diopter. Wavefront-sensing is a promising technology that may lead to highly customized procedures to correct even the slightest corneal aberrations.

There is also some literature on LASIK and PRK performed in atypical or non-ideal situations. Yo, Vroman, Ma, Chao and McDonnell (2000) investigated the outcomes of surgeries performed by inexperienced refractive surgeons. They examined the results of the first PRK procedures performed by 33 ophthalmologists and the first LASIK procedures performed by 19 ophthalmologists, and they found that first-time surgeons were very successful. The authors concluded that PRK and LASIK are safe surgical procedures that are quickly learned and applied by competent ophthalmic surgeons. In 2001, Nassaralla and Nassaralla published a study in which they reported on the results of LASIK performed on nine children ranging from 8 to 15 years old. Normally, an ophthalmologist will wait until a person is at least 18 to 21 years old to use LASIK as a treatment. In this study, LASIK was successfully performed on children with high anisometropia in combination with high myopia or myopic astigmatism. Anisometropia is a condition in which a patient has refractive errors in both eyes that are
significantly different from each other. The results were preliminary, but nevertheless promising.

1.4.3 Side Effects, Complications and Corneal Wound Healing

When excimer laser refractive surgery research began in the late 1980’s and early 1990’s, one of the greatest concerns was the potential harmful photochemical effects of the laser itself. Sliney, Krueger, Trokel and Rappaport (1991) investigated this issue using anesthetized rabbits. They found that photokeratitis, a harmful laser-induced side effect, was non-existent following ablation at clinically relevant fluences ranging from 150-200 mJ/cm$^2$. Only at very high fluences of 10-20 J/cm$^2$ did photokeratitis occur, and even then it seemed to be a result of the subsequent fluorescent emission rather than from the ablating laser light itself.

Another concern related to laser refractive surgery is postoperative contrast sensitivity. This refers to the eye’s ability to perceive contrast and therefore detect movement and detail. Perez-Santonja, Sakla and Alio (1998) studied the contrast sensitivity of fourteen eyes both before and after LASIK for correction of high myopia, ranging from 6.0 to 19.5 diopters. A relatively significant decrease in sensitivity was observed one month postoperatively, but it recovered and had returned to normal by about three months. At six months, corneal sensitivity was actually slightly improved over its original level, but the change was not significant.

Patel, Perez-Santonja, Alio and Murphy (2001) found that LASIK caused a decrease in central corneal sensitivity, probably due to nerve damage, as well as thinning of the tear lipid layer, which may contribute to postoperative dry eye symptoms. The authors hypothesized that a decrease in corneal sensitivity may reduce the efficiency of
the eye’s natural blink response in rebuilding the tear film. However, they also found that the reduction in corneal sensitivity is transient and remedies itself over time.

A considerable literature review is available that describes in detail the types of complications associated with LASIK and how to manage and prevent them (Melki & Azar, 2001). The review covers in detail several flap complications, infection, refractive complications (including central islands and decentration), loss of visual acuity, and keratitis. Flap and microkeratome-related complications are among the most common issues with LASIK. Gimbel et al. (2000a, 2000b) review a number of these complications, including flap wrinkles, dislodged flaps, bleeding, thin flaps, button holes, and corneal perforation, among others. Ruiz-Moreno, Perez-Santonja and Alio (1999) reported on retinal detachment following LASIK, but found that it only occurred in 4 of 1554 eyes studied, an incidence that was no greater than in normal, untreated eyes.

Several studies have been devoted to investigating the formation of topographical features known as steep central islands as a result of excimer laser refractive surgery (Kim & Jo, 2001; Lin, Sutton & Berman, 1993; Noack, Tonnies, Hohla, Birngruber & Vogel, 1997; Oshika, Klyce, Smolek & McDonald, 1998; Shimmick, Telfair, Munnerlyn, Bartlett & Trokel, 1997). A central island is a local area of undercorrection, or reduced ablation, within the larger, relatively uniformly ablated treatment zone. The consensus seems to be that central islands are likely the result of local variations in the ablation rate due to local variations in corneal hydration or liquid pooling. Nevertheless, the direct cause of central islands in the treatment zone remains unproven.

The corneal wound healing response and related issues have been documented by many researchers (Alio et al., 2000; Campos, Cuevas, Garbus, Lee & McDonnell, 1992;
Park & Kim, 1999; Rocha & Schultz, 1996; Schultz, Davis & Eiferman, 1988; Vesaluoma et al., 2000; Wachtlin, Langenbeck, Schrunder, Zhang & Hoffmann, 1999).

It is generally accepted that the wound healing response is much more extensive after PRK than after LASIK, which may account for the greater incidence and degree of corneal haze following PRK. Haze formation, both early and late postoperatively, was studied for PRK and LASIK performed on human patients and linked to the corneal wound healing response (Polunin, Kourenkov, Makarov & Polunina, 1999).

Campos et al. (1992) determined that blowing nitrogen gas over the surface of the cornea during PRK has a number of adverse effects. It causes a slowed healing response, contributes to a higher degree of postoperative corneal haze, and leads to a rougher surface after ablation. These results were only preliminary and were discovered using rabbit corneas rather than human subjects, but are nonetheless intriguing.

1.5 Fundamentals of Laser Ablation and Laser-Tissue Interactions

Much research has been devoted to a better understanding of the mechanisms of the ablation process. A fundamental understanding of ablation and the interactions between the excimer laser and corneal tissue may lead to refinements and improvements in laser refractive surgery.

1.5.1 Overview of Excimer Laser-Corneal Tissue Interactions

Excimer lasers are gas-medium lasers that emit in the ultraviolet range of wavelengths. “Excimer” is a contraction of “excited” and “dimer”, where a dimer is a two-atom molecule. More accurately, a dimer is a diatomic molecule of two identical atoms (e.g., O₂). Hence, it would be more correct to use the term “exciplex”, which would be a combination of “excited” and “diplex” and would correctly reflect the combination of two dissimilar atoms. Nevertheless, the term excimer is widely used
whether the molecule is a true excimer (e.g., F\textsubscript{2} laser) or an actual exciplex (e.g., ArF laser). An excimer laser is realized by application of a high-voltage electrical discharge, typically 15,000-30,000 V, across a chamber that contains an inert gas and a halogen. The discharge is high enough to ionize inert gas atoms, which then react with the surrounding halogen gas to create transient molecules of inert gas ions and halogen atoms. The diatomic molecule forms in an excited state, which readily creates a population inversion due to the instability of the ground state molecule. This excited molecule is the “excited dimer.” The molecule immediately decays to its unstable ground state, which releases an ultraviolet photon, and then dissociates. This process creates a rare two-level laser. The laser of choice for refractive surgery is the argon-fluoride (ArF) excimer laser, which emits at 193 nm (McDonald & Chitkara, 1998).

There are two theories commonly used to explain the mechanics of ultraviolet (UV) laser ablation—thermal and photochemical. Briefly, the thermal model suggests that the laser energy is absorbed by the target and converted into heat energy, which vaporizes the material in a confined local area. Typically, water is considered to be a primary absorber in this case. The photochemical model, known as “ablative photodecomposition” or “cold” ablation, suggests that the energy of a UV laser is sufficient to break chemical bonds and photochemically decompose the material (Dyer & Srinivasan, 1986; Garrison & Srinivasan, 1984; Hahn, Ediger & Pettit, 1995; McGrann, Neev & Berns, 1992; Pettit & Sauerbrey, 1993; Srinivasan, 1989; Tokarev, Lunney, Marine & Sentis, 1995; Trokel, Srinivasan & Braren, 1983; Vogel & Venugopalan, 2003; Yeh, 1986). The ArF excimer laser has a photon energy of 6.4 eV, while the dissociation energies of C-C bonds and C-
H bonds are 3.6 eV and 4.3 eV, respectively. The “loose” material is then rapidly ejected from the surface, in what is called an ablation plume, as a pressure wave expands.

1.5.2 Early Research on Excimer Laser Ablation of Polymers

Much of the early ablation research was done using polymers as surrogates for corneal tissue (Ball et al., 1995a, 1995b; Burns & Cain, 1996; Chirila & Van Saarloos, 1992; Costela et al., 1995; Davis & Gower, 1987; Ediger & Pettit, 1992; Ediger & Pettit, 1993; Ediger et al., 1993a, 1993b; Hahn, Pettit & Ediger, 1994; Hansen, 1989; Hopp, Csete, Szabo & Bor, 1995; Noack et al. 1997; O’Donnell, Kemner & O’Donnell, Jr., 1996a, 1996b; Pettit, Ediger, Hahn, Brinson & Sauerbrey, 1994; Srinivasan, 1993; Srinivasan, Braren, Dreyfus, Hadel & Seeger, 1986; Srinivasan, Braren & Dreyfus, 1987; Srinivasan & Braren, 1989; Srinivasan, Braren, Casey & Yeh, 1989; Srinivasan, Braren & Casey, 1990). Several important conclusions were reached based on this large body of research.

The laser radiation must reach a threshold fluence, or energy per unit area, to achieve ablation. Below the threshold, no ablation will occur. Above the threshold, ablative photodecomposition occurs and material is etched away. Excimer laser radiation at 193 nm ablates material locally without affecting or melting the surrounding material, while the ablated material is ejected supersonically (Garrison & Srinivasan, 1984). It was found that ablation of polyimide at high laser fluence begins within 4-6 nanoseconds (ns) of the start of the laser pulse and that a high-pressure compression wave exceeding $10^7$ Pascals (Pa) is created (Dyer & Srinivasan 1986).

Ablation of polymethyl methacrylate (PMMA) was studied by Hopp et al. (1995) and Srinivasan (1993) using high-speed photography. Both studies found a delay between the laser pulse and the beginning of material ejection that was long relative to
the width of the laser pulse. Specifically, a typical excimer laser pulse is only 10-20 nanoseconds in width, while the ejection of material did not begin until hundreds of nanoseconds later. Noack et al. (1997) found that the vortices produced by the ablation plume dynamics may redeposit some of the ejected material near or at the center of the ablation zone and lead to the formation of central islands. Studies in which the ablation rate of polymers was measured using a variety of methods generally found that the ablation rate increases with increasing fluence up to a saturation point where the ablation rate becomes relatively constant (Chirila & Van Saarloos, 1992; Costela et al., 1995).

In more applied studies, researchers found that polyimide displays a transient decrease in reflectivity during the trailing edge of the laser pulse while also displaying enhanced transmission with increasing laser fluence (Ball et al., 1995a, 1995b; Hahn et al., 1994; Pettit et al., 1994). Ediger et al. (1993a, 1993b) showed that the transient decrease in reflectivity of polyimide during ablation was likely not due to scattering of light by the ablation plume, although this theory was popular (Davis & Gower 1987). This invalidates the idea that scattering of light by the ablation plume is responsible for a transient decrease in reflectivity during the laser pulse.

There is a fundamental difference between PMMA and polyimide that should be noted. Although there are ester side groups, the main structure of PMMA is a linear hydrocarbon chain and PMMA contains no nitrogen atoms. In contrast, polyimide contains nitrogenated ring structures and amide groups, both of which resemble structures present in collagen. For this reason, the ablation of polyimide is likely to be more representative of corneal tissue ablation.
1.5.3 Corneal Tissue Ablation Research

Fundamental studies have been done to investigate the physics of excimer laser ablation of the cornea, as well as the histological effects of the radiation, the uniformity of the ablated surface, and the ablated material itself (Al-qahtani, McLean, Weiblinger & Ediger, 2001; Berns et al., 1999; Fantes & Waring, 1989; Kermani, Koort, Roth & Dardenne, 1988; Kitai et al., 1991; Krueger & Trokel, 1985; Puliafito et al., 1985, 1987a, 1987b; Telfair et al., 2000; Trokel, 1983). High-speed photographic images of the ablation process showed that a small plume of ablated material extending about 0.2 mm from the corneal surface is present about 0.5 microseconds (µs) after the laser pulse, and that by 50 µs there is a prominent mushroom cloud with a long, fully-developed stem (Puliafito et al., 1987a). The authors concluded that the actual ablation process is over somewhere between 5 and 15 µs after the laser pulse, but that the material that is ejected continues to travel away for at least 150 µs. In general, most of the studies agree that the excimer laser is safe and effective for ablation of corneal tissue, that there is clearly a threshold laser fluence required for ablation to occur, and that the results may be highly repeatable and predictable when using a laser fluence much higher than the threshold because of an ablation rate saturation effect.

It has been seen experimentally that high laser fluences may cause acoustic shock waves and secondary radiation, and high pulse repetition rates can cause thermal side effects (McGrann et al., 1992). Early on, it was widely thought that ablation was a strong thermal process that led to vaporization of much of the irradiated material. A laser light scattering study was done on the ablation plume to investigate the plume particles (Hahn et al., 1995). Scattering of water spheroids was measured and it was determined that
these particulates accounted for 78% of the total ablated material, which corresponds very closely to the water content of cornea (approximately 75%). This finding suggested the absence of any significant thermal process or water vaporization, and supported the photochemical model of ablation.

An alternative view, presented by Staveteig and Walsh (1996), is that water in corneal tissue may be superheated by the excimer laser and that this superheated water may subsequently become a very strong absorber of the laser light. This is discussed in greater detail in Section 1.8 of this chapter. The possibility that excimer laser ablation is thermally driven was further supported by Ishihara et al. (2002). Using thermal radiation measurements, they determined that the peak surface temperature of porcine corneal tissue, coinciding with the end of the ablating laser pulse, was approximately 240°C at clinically relevant laser fluences. However, this peak temperature is highly transient and may not be representative of the true corneal temperature during ablation. The idea that the instantaneous peak surface temperature of the cornea determines the overall nature of the ablation process may be overly simplistic.

Another study showed, using photomicrographs to image ablation sites in calf eyes, that stromal integrity remains intact in areas immediately adjacent to ablation zones and that the ablation threshold fluence is independent of the pulse repetition rate. The same study also found that at lower fluences, below the ablation threshold, there was no coagulation of the tissue (Krueger, Trokel & Schubert, 1985). This supports the idea that ArF excimer laser ablation at 193 nm is a photochemical process.
1.5.4 Spectroscopic Studies

There have been a number of spectroscopic studies performed for excimer laser ablation of polymers (Davis, Gower, Fotakis, Efthimiopoulos & Argyrakis, 1985; Koren & Yeh, 1984a, 1984b; Srinivasan et al., 1986). Davis et al. (1985) ablated PMMA samples using a laser fluence of approximately 300 mJ/cm$^2$ and recorded spectra of the natural emission following the ablation event. They noted the presence of prominent emission bands which were believed to correspond to the species N$_2$, CO, C$_2$, CH, and CN, and they concluded from their experiments that PMMA ablation is primarily a photochemical process in which polymeric bonds are directly broken. Srinivasan et al. (1986) also studied PMMA, but used laser-induced fluorescence (LIF) techniques. In this study, LIF with a pumped dye laser was used to probe C$_2$ in the ablation plume, etch depths were measured with a step profilometer, and volatile ablation products were trapped and analyzed using a gas chromatograph-mass spectrometer (GC-MS). It was found that the major products of 193-nm ablation of PMMA are C$_2$, methyl methacrylate (MMA), and an unknown species with a relatively low molecular weight, and evidence from these experiments further suggested that the ablation process is photochemical in nature. The major limitation of these experiments is that very little information is offered concerning the time gate of the measured emission. Emission and fluorescence are inherently highly time-dependent, and critical timing parameters are not discussed in these studies.

Koren and Yeh (1987a, 1987b) investigated the emission spectra from various polymers, including polyimide and PMMA, exposed to excimer laser radiation. For both PMMA and polyimide, they observed bands believed to correspond to C$_2$, C, and CN, and they concluded from their experiments that laser ablation of polymeric materials is a
statistical thermodynamic process. As with the PMMA experiments detailed above, these studies provided little or no information about the gate timing for the emission collection. Furthermore, in some cases, the laser fluences required to distinguish the discrete emission bands from the continuum emission far exceeded clinically relevant values.

Researchers have also investigated the ablation of corneal tissue using spectroscopic methods (Cohen, Chuck, Bearman, McDonnell & Grundfest, 2001; Ediger, 1991; Lubatschowski et al., 1994). Ediger (1991) measured prompt fluorescence, integrated over 100 ns, from enucleated rabbit corneas subjected to excimer laser radiation at low fluence (~85 mJ/cm$^2$) and high fluence (~950 mJ/cm$^2$). Investigating a spectral window spanning from 250 to 500 nm, two broad fluorescence bands were observed for low fluence exposure, while only the stronger of the two bands was obvious for high fluence exposure. These results were highly qualitative in nature and no significant conclusions were reached.

Lubatschowski et al. (1994) measured fluorescence from porcine corneal tissue, with the fluorescence signal integrated over 1 µs beginning immediately after the end of the ablating laser pulse. At very high laser fluence exposure (2 J/cm$^2$), emission bands were observed that were attributed to C, Na, H, N, and O. However, only a single very broad fluorescence band was observed between 200 and 800 nm when the corneas were exposed to more clinically relevant laser fluences below 500 mJ/cm$^2$. The discrete emission peaks at high laser fluence were believed to be the result of a weak laser-induced plasma. Cohen et al. (2001) performed an ablation study on excised human corneas (obtained from an eye bank) in which they measured emission spanning 200 to 900 nm for a laser fluence of approximately 170 mJ/cm$^2$. As with the rabbit experiments
described above (Ediger, 1991), the results in this study were qualitative and inconclusive. The researchers performed a complicated principal components analysis on the data, yet they provide few actual spectra and do not assign the features to any particular species, and there is no information provided about the timing of the measured signal.

Overall, the spectroscopic studies that have been performed thus far, both for polymers and corneal tissue, have been mostly qualitative and inconclusive. In some cases, useful spectra were only obtained at laser fluences well beyond the realm of clinical relevance. In other cases, little or no information has been provided concerning the timing of the measured signals, which is critical when dealing with fluorescence and emission spectroscopy. More careful spectroscopic studies, including natural emission as well as laser-induced fluorescence, are expected to provide insight into the ablation process and the transient species involved and are therefore an important aspect of current and future work.

1.6 Quantification of the Corneal Tissue Ablation Rate

Perhaps the most important parameter when considering excimer laser refractive surgery, and probably the least characterized, is the ablation rate of the material itself. The ablation rate of any material can be measured by exposing it to repeated laser pulses and then dividing the total depth of the resulting ablation crater by the total number of laser pulses. This presents a problem, however, due to difficulties in accurately measuring the depth of the crater. Several different methods of measuring the depth have been suggested and used, though their accuracy and precision are not always quantified in the literature.
Aron-Rosa et al. (1986) found that the ablation depth increases with increasing laser fluence for cadaverous corneal tissue by measuring cross-sections of ablation craters with a light microscope. Another method commonly used is to focus a high magnification light microscope on the pristine corneal surface of an eye, then measure with a micrometer the distance the microscope must travel to focus on the bottom surface of the crater (Van Saarloos & Constable, 1990). This procedure has been performed with organic polymers such as PMMA and polyhydroxyethyl methacrylate (PHEMA) (Chirila & Van Saarloos, 1992; Costela et al., 1995). This procedure, however, is difficult to perform for eyes because of their inherent curvature.

Srinivasan et al. (1986, 1987) measured ablation depths in PMMA and polyimide using a Tencor Alpha step profilometer, and Shimmick et al. (1997) observed ablation depth profiles using an optical profilometer that projected high contrast silhouette images onto a CCD camera. Lubatschowski, Kermani, Welling and Ertmer (1998) used a more direct approach and measured the ablation depth for PMMA wafers with a pachymeter. While profilometers are very applicable to solid polymers, they are unsuited for “soft” corneal tissue, as the stylus will gouge into the tissue and thereby fail to follow the actual crater profile.

While all of the above mentioned techniques provide ablation rate data, notwithstanding the limitations of profilometry for tissue, the precision of ablation crater depth and ablation rate measurements is a key issue if small variations in ablation rate are to be measured. Campos et al. (1993) determined the ablation rate of porcine corneal tissue to be 0.27 µm/pulse at a fluence of 180 mJ/cm² based on the method of recording the number of laser shots required to achieve perforation of a sample of known thickness.
(either a section of corneal tissue or the full thickness of an excised cornea). Similarly, Fuxbruner et al. (1990) measured the ablation rate of corneal tissue for rabbits at various laser fluences using a shots-to-perforation scheme, and reported a value of approximately 0.22 µm/pulse at 80 mJ/cm². One of the main issues with the shots-to-perforation method is that it measures the average ablation rate over the entire thickness of the material, while the ablation rates of the anterior and posterior portions may differ significantly.

Using Scheimpflug videography to measure in vivo the curvature of human corneas before and after ablation, Huebscher, Genth and Seiler (1996) measured ablation rates ranging from 0.23-0.30 µm/pulse. A limitation of this method is the assumption that changes in corneal curvature from the beginning to the end of ablation are due only to removal of tissue, although the curvature may in fact be affected by changes in corneal hydration as well. Dougherty, Wellish and Maloney (1994) measured the ablation rate in bovine corneal tissue by calculating the difference in weight of the tissue samples before and after ablation and correcting for dehydration due to evaporation using control samples. The accuracy and precision of this method is difficult to assess due to the many inherent assumptions, including that the amount of dehydration is constant for different eyes. To convert the mass of tissue ablated to an ablation depth per laser pulse, it was necessary to assume an average value for the density of all bovine corneal tissue, although transient dehydration may be present. Clearly there are many issues associated with the methods described above for determining corneal ablation rates, including questions of accuracy, precision, and experimental complexity.
As an alternative to the above techniques, previous studies have examined the use of white-light interferometry to quantify the roughness of the cut surface created during laser ablation of PMMA (O’Donnell, 1996a, 1996b). Rings, Sievers and Jansen (1999) used a white-light interferometer to examine the shapes and sizes of craters created by laser ablation of PMMA. White-light interferometry, however, is not directly applicable to corneal tissue ablation craters due to the “deformable” nature of tissue, the susceptibility to tissue dehydration or expansion, and the transparent nature of corneal tissue.

The average ablation rate of corneal tissue was determined empirically in early clinical trials and is routinely used in clinical laser refractive procedures. However, there is no method for predicting the true ablation rate for a given patient because the exact nature of the mechanisms involved in excimer laser ablation are not well characterized or understood. Hence, further understanding of the ablation mechanisms is a key focus of the current work.

1.7 Role of Corneal Hydration in Excimer Laser Ablation

1.7.1 Corneal Hydration and Ablation Rate

Notwithstanding the difficulties of measuring ablation rates, some general trends have been observed. The ablation rate is a function of the laser fluence, which is the laser pulse energy per unit exposure area, commonly expressed in units of millijoules per square centimeter (mJ/cm²). Furthermore, the ablation rate is generally accepted to correlate with the degree of corneal hydration. Lin et al. (1993) stated outright that moist corneal tissue ablates less than dry tissue. It is reported that changes in corneal hydration may result in up to 10-15% error in refractive correction (Dougherty et al., 1994; McDonald & Chitkara, 1988).
In an effort to understand this, Dougherty et al. (1994) investigated various ablation rates, including the hydrated tissue (i.e., wet) ablation rate and what they referred to as the dry component ablation rate (defined as the mass of collagen and ground substance removed per surface area per laser shot). They reported that the dry component ablation rate increased with decreasing hydration, and that the wet tissue ablation rate decreased with decreasing hydration. The latter observation is consistent with the earlier published work (Waring, 1989) and was supported by a later ablation study of hydrogel polymer materials in varying hydration states (Feltham, Optom & Stapleton, 2002). Very recently, the authors of a statistical LASIK outcomes study concluded that corneal hydration affects the ablation rate after observing a statistical dependence of refractive results on humidity (Walter & Stevenson, 2004). Overall, researchers are in broad agreement that the degree of corneal hydration can directly affect the ablation rate of corneal tissue with 193-nm excimer laser radiation. However, the exact nature of the relationship is not widely agreed upon and no sound theoretical framework exists.

To elucidate the dynamic nature of corneal hydration, as well as to quantify factors potentially affecting corneal hydration during clinical procedures, a quantitative measurement of hydration is necessary. Pallikaris et al. (1998) investigated the use of laser-induced breakdown spectroscopy (LIBS) to monitor corneal hydration. They reported that the atomic emission intensity of hydrogen remained constant while the atomic emission of calcium varied with corneal hydration. The LIBS technique entails the use of a moderate laser pulse energy (tens of millijoules) to vaporize a small sample of corneal tissue, resulting in a strong shock wave and visible radiative emission. The
destructive nature of LIBS is a significant limitation for *in vivo* analysis, and therefore prevents integration into clinical studies supporting refractive procedures.

1.7.2 Quantifying Corneal Hydration using Raman Spectroscopy

Raman spectroscopy is well suited as a technique for measuring and monitoring corneal hydration and has been investigated in several recent studies. Raman spectroscopy uses a single-wavelength excitation source (typically a low-power continuous laser beam) to probe the vibrational bond energies of constituent molecules through an inelastic light scattering process known as the Raman effect. It is minimally invasive, making it possible for integration into clinical investigations and systems.

The use of Raman spectroscopy for the study of biological tissues has been well documented. It has been studied for its potential to differentiate normal and dysplastic or cancerous tissues with no collateral tissue damage (Frank, McCreery & Redd, 1995; Gniadecka et al., 1997a, 1997b; Kline & Treado, 1997; Lawson, Barry, Williams & Edwards, 1997; Manoharan et al., 1998; Pappas, Smith & Winefordner, 2000; Redd, Feng, Yue & Gansler, 1993; Schut et al., 2000; Ullas et al., 1999; Yazdi et al., 1999). Researchers have even outlined a strategy for the use of isotope-edited Raman spectroscopy to probe peptide bonds in the proteins of biological tissues (Dong et al., 2001).

The cornea is readily amenable to analysis by Raman spectroscopy due to its composition. Collagen and other proteins that are prominent in corneal tissue have characteristic vibrational frequencies, known as Raman bands or Raman shifts. Most notably, the band at 2940 cm$^{-1}$ corresponds to the C-H stretching vibration, which is characteristic of the numerous C-H protein bonds in corneal tissue. Water, which constitutes about 75% of normal corneal tissue, displays a broad Raman band
corresponding to the O-H stretching vibration in the region from 3000 to 3700 cm\(^{-1}\) (which is commonly considered to be centered at approximately 3400 cm\(^{-1}\)).

Jongsma et al. (1997) describe the use of a confocal Raman system for \textit{in vitro} study of rabbit eyes. This system involves excitation and collection of Raman scattered light through a confocal microscope objective. The confocal implementation has the advantage of enhanced spatial surface and depth resolution, thereby reducing the size of the probe volume. This enhanced resolution reduces the effects of potential background fluorescence and spectral noise, and therefore improves the signal-to-noise ratio.

The confocal Raman system, because of the very small probe volume and high depth resolution, enables investigation of the hydration gradient through the depth of the cornea. Using the same confocal Raman system discussed above, Bauer et al. (1998) measured corneal hydration in both \textit{in vitro} and \textit{in vivo} rabbit corneas, as well as in collagen-based phantom media. They reported, using the ratio of the water O-H stretch Raman intensity to the C-H stretch intensity as a metric, that hydration was constant throughout a sample of albumin with a uniform water distribution. These findings suggest that the measured hydration is a true function of physical hydration and is independent of the probe depth, even in samples with non-uniform water distribution, such as corneal tissue. In a more recent study, Bauer, Hendrikse and March (1999) found that the relative hydration of the anterior surface of the human cornea \textit{in vivo} decreased with the application of the dehydrating drug Muro 128. This important study demonstrated the utility of confocal Raman spectroscopy for measuring corneal hydration, and it was the first time that \textit{in vivo} Raman spectra were successfully obtained from human corneal tissue.
1.8 Optical Properties of Cornea and Corneal Constituents

1.8.1 Water and Saline Solutions

It has been suggested that variations in ablation rate may exist because of transient changes in the optical properties of corneal tissue or its constituents. Water may act as a strong chromophore for 193-nm laser light, which may explain why the ablation rate is so closely related to corneal hydration. Staveteig and Walsh (1996) investigated the dynamic absorption of ArF excimer laser light by water, and found that superheated water (due to absorption of 2.94-µm light from an Erbium:YAG laser) strongly attenuated excimer laser light. They determined that the absorption coefficient of water increased by as much as five orders of magnitude depending on the fluence of the Er:YAG exposure (i.e., the degree to which water was superheated). However, water in corneal tissue is unlikely to be superheated during excimer laser ablation, so this study may not be clinically relevant. It may be possible that confinement of corneal tissue during the ablation event may result in pressure buildup and localized superheating, but no experimental evidence has directly supported this mechanism.

As a reference, the absorption coefficient of water is plotted in Figure 1-4 based on information from several sources (Buitevald, Hakvoort & Donze, 1994; Hale & Querry, 1973; Kopelevich, 1976; Querry, Cary & Waring, 1978; Quickenden & Irvin, 1980; Segelstein, 1981). Clearly, there is a steep gradient in the absorption below a wavelength of approximately 200 nm, as the value changes by several orders of magnitude over only a few nanometers. Variations in the measurements are likely due to differences in water composition. Nevertheless, there appears to be agreement that the absorption coefficient of water with respect to 193-nm radiation is only approximately 0.1-0.2 cm\(^{-1}\).
Figure 1-4. Absorption coefficient of water as a function of wavelength, plotted on a log-linear scale, as obtained from several sources.

Dair, Ashman, Eikelboom, Reinholz and Van Saarloos (2001) studied the absorption of 193-nm laser light by sodium chloride and balanced salt solutions, because it was believed that surface fluid might absorb excimer laser light and lead to less ablation than expected. The 193-nm absorption coefficient of balanced salt solution was measured as 140 cm$^{-1}$, while the absorption coefficient of 0.9% sodium chloride solution was measured as 81 cm$^{-1}$. This may be an issue, because salt solutions are often placed on the surface of the eye during refractive surgeries, and these fluids may inhibit ablation. In a similar study, Michalos, Avila, Florakis and Hersh (1994) measured the transmission of UV light through human tears. Although they did not investigate ArF 193-nm excimer laser light, they measured only 60% transmission of 195-nm light.
1.8.2 Corneal Tissue

Many of the existing models of excimer laser ablation are coupled strongly to the corneal tissue absorption coefficient, which for 193-nm excimer laser light is perhaps one of the largest sources of controversy and the focus of much research. One of the first values reported for the tissue absorption coefficient, determined by measuring transmission of excimer laser light through thin human and bovine corneal sections, was $2700 \text{ cm}^{-1}$ at 193 nm (Puliafito et al., 1985). This value was reiterated and further supported by subsequent research (Puliafito et al., 1987b), including a similar study with human and porcine cornea that yielded an average value of $2410 \text{ cm}^{-1}$ (Lemmares, Hu & Kalmus, 1997).

However, by the mid-1990’s, researchers began to reexamine this issue. Bor et al. (1993) reported a value of $20,370 \text{ cm}^{-1}$ for porcine corneal tissue based on fitting a logarithmic curve to a plot of ablation rate vs. laser fluence measurements. By directly measuring corneal tissue reflectance and applying Fresnel theory to determine the complex refractive index of the tissue, Pettit and Ediger (1996) reported an absorption coefficient of approximately $40,000 \text{ cm}^{-1}$ for bovine cornea. Yablon, Nishioka, Mikic and Venugopalan (1999) measured the absorption coefficient of bovine cornea using a novel technique called interferometric photothermal spectroscopy and reported a value of $19,000 \text{ cm}^{-1}$.

The values reported by researchers in more recent literature are an order of magnitude larger than the absorption coefficient first reported by Puliafito et al. (1985). Pettit and Ediger (1996) contend that the absorption coefficient must be on the order of $20,000 \text{ cm}^{-1}$ based on theoretical considerations, namely by assuming that the peptide bond is the primary chromophore in corneal tissue for 193-nm light and translating the
bond density into an absorption coefficient, as well as in light of experimental evidence based on measured ablation rate values as a function of laser fluence. However, consideration of a single absorption coefficient makes the implied assumption of a static and unchanging value during the ablation event, which may be an oversimplification.

Many researchers have suggested that the absorption coefficient is actually a dynamic quantity that may be enhanced under ablative conditions, as well as be influenced by matrix effects such as tissue hydration. It is generally accepted that there is a static “small-signal” absorption coefficient that remains constant for laser fluences below the ablation threshold. Some research has shown, however, that there are transient decreases in both corneal tissue reflectivity and transmission through the tissue under ablative conditions (Ediger et al., 1993a, 1993b; Pettit & Ediger, 1993; Pettit et al., 1993, 1995). Such data suggest that there is a transient effect that dynamically changes the absorption coefficient of corneal tissue during the ablation process, notably during the time course of the excimer laser pulse. Furthermore, research suggests that tissue hydration may play a role in transient changes in tissue absorptivity, possibly due to direct absorption of laser energy by water molecules and subsequent vaporization (Pettit et al., 1995; Staveteig & Walsh, 1996; Tsunoda et al., 2001).

Alternative views propose that ablation events at high laser fluence induce radical species formation with very high absorption cross-sections, while also causing boiling of the water fraction of cornea, resulting in changes in the overall absorptivity of the tissue (Kitai et al., 1991). Electron paramagnetic resonance studies have corroborated the idea that reactive free radical species are created during excimer laser ablation, suggesting that these radicals may have a significant role in laser-tissue interactions (Pettit et al., 1996).
Despite the large body of research to date, no work has systematically investigated
the absorption properties of individual corneal tissue constituents (namely collagen and
water) using direct measurements. It has been commonly suggested or assumed that the
peptide bond in collagen is the primary chromophore for 193-nm light (Coohill, 2002;
Pettit & Ediger, 1996; Wetlaufer, 1962), but this idea has not yet been experimentally
validated. Furthermore, no clear and comprehensive description of the corneal tissue
laser absorption and ablation processes has been developed. The current work is intended
to further elucidate the role of corneal constituents in the ablation process.

1.9 Current State of Modeling for Excimer Laser Ablation of Corneal Tissue

There have been a number of literature reviews that address the mechanisms and
chemistry of excimer laser tissue ablation (Manns, Milne & Parel, 2002; Paltauf & Dyer,
2003; Vogel & Venugopalan, 2003) as well as the physics of polymer ablation
(Srinivasan & Braren, 1989). A careful review of the literature, however, reveals the lack
of a comprehensive model that can be used to explain or predict tissue ablation rates with
a high degree of accuracy. Many have suggested, and some research has supported, the
idea that corneal tissue absorbs 193-nm ArF excimer laser light according to the Beer-
Lambert law, meaning the intensity of the light decays exponentially with depth into the
tissue (Manns et al., 2002; McGrann et al., 1992; Paltauf & Dyer, 2003; Pettit &
Sauerbrey, 1993; Vogel & Venugopalan, 2003). The Beer-Lambert law is described by:

\[ I(x) = I_0 e^{-\alpha x} \]  

(1-1)

In this equation, \( I_0 \) is the laser intensity incident at the tissue surface, \( I(x) \) is the laser
intensity after penetrating to tissue depth \( x \), and \( \alpha \) is the tissue absorption coefficient
(usually reported in \( \text{cm}^{-1} \)) for the particular wavelength of laser light. Beer-Lambert
behavior is certainly a valid approach for low-power irradiation, where the incident energy density is well below the ablation threshold.

However, under ablative conditions, non-linear optical effects may alter the nature of the absorption coefficient. Notwithstanding such effects, the Beer-Lambert law can be used to provide a first-order estimate of ablation depth in what is often referred to as a “blow-off” model. In this model, the depth of tissue ablation for a single laser pulse is equal to the depth at which the laser intensity has decayed to the threshold required for ablation. Defining $I_{th}$ as the ablation threshold intensity, with incident intensity $I_o$, the blow-off model predicts an ablation depth equal to:

$$d_{ablation} = \frac{1}{\alpha} \ln\left(\frac{I_o}{I_{th}}\right)$$  \hspace{1cm} (1-2)

Ablation, as predicted by the blow-off model, is coupled strongly to the corneal tissue absorption coefficient. Ablation research, therefore, would benefit tremendously from a better understanding of the tissue absorption coefficient and its temporal behavior under ablative conditions.

Other analytical models have been used in an attempt to explain UV laser ablation of corneal tissue. They include but are not limited to thermal models, single-photon absorption, multiphoton absorption, and chromophore saturation. Some models attempt to explain the ablation kinetics by including the effects of absorption in the plume (Tokarev et al., 1995). A model was developed to include the effects of chromophore saturation and multiphoton absorption, and this model correctly explained experimental data for a variety of materials and a range of UV laser wavelengths (Pettit & Sauerbrey, 1993).
Sutcliffe and Srinivasan (1986) developed a dynamic model that related the time evolution of the ablation process to experimental parameters such as laser fluence, wavelength, and width of the laser pulse. While their model predicted the ablation rate of polymeric materials with reasonable accuracy, it failed to include any effects of hydration or other effects that may be present in corneal tissue. It is clear that no model has yet been able to completely explain or predict the tissue ablation process.

1.10 Objectives of Present Research

The research presented here supports the following objectives:

1. To quantify the ablation rate of corneal tissue with a precise and reliable measurement technique, and measure ablation rate as a function of laser fluence.

2. To develop a method for determining the ablation rate *in situ* and in real time for eventual integration into a clinical feedback system.

3. To gain further understanding of the ablation process itself, namely the mechanisms of excimer laser-corneal tissue interactions and the parameters (e.g., corneal hydration) that affect ablation.

4. To integrate the results of all experiments and develop a comprehensive model for excimer laser corneal ablation
CHAPTER 2
EXPERIMENTAL FACILITIES AND MEASUREMENT AND ANALYSIS TECHNIQUES

The present work encompasses a large and varied body of experiments, requiring multiple lasers and using several optical collection geometries. Some of the techniques used include time-resolved reflectance, confocal Raman spectroscopy, white-light interferometry, and time-resolved emission spectroscopy. This chapter is intended to provide a detailed statement of the experimental methods and data analysis procedures. To assist in this description, illustrative examples are given and are accompanied by explanations of experimental details. Full treatment of these experiments and corresponding results are included in the remainder of this dissertation.

The major equipment used throughout the course of the experiments is listed in Table 2-1. Nearly all of the work was done using the Alcon ArF excimer laser, which is a laboratory version of the clinical LADARVision laser system, the oscilloscope, and two phototubes, each powered by a high-voltage DC power supply. An electro-optic shutter was used to remotely control and count the delivery of laser pulses to the target of interest while allowing the laser to operate continuously. The ArF excimer laser made by GAM Laser, Inc. was introduced for time-resolved emission studies, and was used in conjunction with the intensified CCD and the spectrometer. This laser was controlled using a PC and manufacturer-provided software, which inherently controlled and counted the delivery of laser pulses, making an external shutter unnecessary. All of the remaining
equipment, including optical components such as mirrors and filters, is detailed in the sections corresponding to the relevant experiments.

Table 2-1. Major experimental equipment.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Manufacturer (Model or Part #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArF excimer laser</td>
<td>193 nm, 10 ns (FWHM), Max 5 mJ/pulse, Max 100 Hz</td>
<td>Alcon (N/A)</td>
</tr>
<tr>
<td>ArF excimer laser</td>
<td>193 nm, 10 ns (FWHM), Max 12 mJ/pulse, Max 250 Hz, Low jitter</td>
<td>GAM Laser (EX5)</td>
</tr>
<tr>
<td>Laser shutter</td>
<td>Electro-optic shutter, 8-mm aperture, Max 5 Hz processing</td>
<td>NM Laser Products (LST400)</td>
</tr>
<tr>
<td>Digital oscilloscope</td>
<td>2.5 gigasamples/second, 2 channels, 8-bit vertical resolution</td>
<td>LeCroy Corp. (9361)</td>
</tr>
<tr>
<td>Biplanar phototube</td>
<td>270-ps rise time, 100-ps fall time, 185-650 nm spectral response</td>
<td>Hamamatsu Corp. (R1193U-02)</td>
</tr>
<tr>
<td>High-voltage DC power supply</td>
<td>Max 2.5 kV output, Max 10 mA current</td>
<td>Stanford Research Systems (PS325)</td>
</tr>
<tr>
<td>Digital delay generator</td>
<td>2 pulse outputs, Max delay 1000 s, Max trigger rate 1 MHz</td>
<td>Stanford Research Systems (DG535)</td>
</tr>
<tr>
<td>Spectrometer</td>
<td>0.275-meter spectrometer, 1200 or 2400 grooves/mm</td>
<td>Acton Research Corp. (SpectraPro-275)</td>
</tr>
<tr>
<td>iCCD camera</td>
<td>Intensified charge-coupled device, 1024 x 256 imaging array</td>
<td>Princeton Instruments (1024-MLDS-E1)</td>
</tr>
<tr>
<td>iCCD pulse generator</td>
<td>Programmable pulse generator for CCD gate timing</td>
<td>Princeton Instruments (PG-200)</td>
</tr>
<tr>
<td>Raman spectrometer</td>
<td>Confocal micro-Raman system, 632.8-nm He:Ne laser, CCD detection</td>
<td>Jobin Yvon (LabRam Infinity)</td>
</tr>
<tr>
<td>White-light interferometer</td>
<td>Vertical scanning optical 3D profiler, Manual x/y stage, Tip/tilt control</td>
<td>Veeco Instruments (Wyko NT1000)</td>
</tr>
</tbody>
</table>

2.1 Time-Resolved Reflectance Measurements

These measurements were performed in an effort to quantify differences in the waveform shapes of the incident laser and reflected light pulses due to variations in relevant parameters, including corneal hydration and laser fluence. The waveform shapes are the temporal profiles of the incoming (incident) laser pulse and the portion of the laser light that is immediately reflected from the target.
2.1.1 Experimental Equipment and Configuration

The configuration for these experiments is shown in Figure 2-1. The incident laser pulse waveform was obtained by aligning a UV-grade quartz beamsplitter (wedge) at approximately 45° with respect to the beam path. The wedge reflected approximately 5% of the excimer laser beam as a reference beam, and the relatively low-intensity reflection from the wedge was passed through a 193-nm interference filter and directed onto a photodetector. This reference beam, lower in intensity than the original laser beam but with the same waveform shape, was recorded as the incident pulse.

The laser light transmitted through the quartz wedge was directed through a pierced mirror to the target. Light reflected, both specularly and diffusely, from the surface of the eye during the ablation event was reflected by the pierced mirror, then focused by a lens through a 193-nm interference filter and onto a second photodetector. The signals from

Figure 2-1. Experimental configuration for time-resolved reflectance experiments.
both photodetectors were fed into the digital oscilloscope and saved for processing. Neutral density filters were used to attenuate both the incident and reflected beams to ensure signal linearity and prevent detector saturation, thereby preserving the true waveform shape.

Table 2-2 contains a full listing of the collection optics for these experiments. It should be noted that the laser used in these experiments was the Alcon excimer laser listed in Table 2-1. The photodetectors used were the Hamamatsu biplanar phototubes, the signals were recorded with the LeCroy digital oscilloscope, and the delivery of laser pulses was controlled using the electro-optic shutter, all of which are also listed in Table 2-1. Depending on the exact nature of the experiments, the ablation target ranged from a glass microscope slide to an organic polymer (e.g., PMMA) to a whole bovine eye globe. Preliminary experiments were done using microscope slides and polymers, while the bulk of the meaningful experiments were done with bovine corneal tissue.

Table 2-2. Listing of equipment for time-resolved reflectance experiments.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Manufacturer (Model or Part #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz wedge</td>
<td>1° large wedge, 2.0” diameter, 0.5” thickness, UV-grade fused silica</td>
<td>CVI Laser Corp. (LW-1-2050-UV)</td>
</tr>
<tr>
<td>193-nm mirror</td>
<td>2.0” diameter, 0.375” thickness, Min 97% reflectance</td>
<td>CVI Laser Corp. (ARF-2037-45-UNP)</td>
</tr>
<tr>
<td>Pierced mirror</td>
<td>2.0” diameter, 0.375” thickness, Min 85% reflectance, 3/8” hole drilled at 45° through center</td>
<td>CVI Laser Corp. (PAUV-PM-2037-C)</td>
</tr>
<tr>
<td>Focusing lens</td>
<td>Plano-convex lens, 50.8-mm diameter, 75-mm focal length, UV-grade fused silica</td>
<td>CVI Laser Corp. (PLCX-50.8-38.6-UV)</td>
</tr>
<tr>
<td>Neutral density (ND) filters</td>
<td>2.0” diameter, 1/16” thickness, UV-grade fused silica, Optical densities of 0.3, 0.6, 1.0, 2.0</td>
<td>Optics for Research (FDU-0.3,0.6,1.0,2.0)</td>
</tr>
<tr>
<td>193-nm interference filters</td>
<td>25-mm diameter, 15 ± 5 nm (FWHM), Min 12% peak transmittance</td>
<td>Melles Griot (03 FIU 101)</td>
</tr>
</tbody>
</table>
2.1.2 Cross-Correlation Functions of the Incident and Reflected Waveforms

A typical ArF excimer laser pulse has a full width, from edge to edge, of approximately 15 to 20 nanoseconds (ns). Under ablative conditions, the reflected pulse typically shows a marked decrease in intensity over the latter half of the pulse, on the trailing edge of the peak. Figure 2-2 shows the incident and reflected waveforms for a laser pulse striking the surface of a glass microscope slide, where the laser fluence is below the ablation threshold (i.e., sub-ablative). Figure 2-3 shows the waveforms for a laser pulse as it ablates the surface of a normally hydrated bovine eye.

In the case of the ablating laser pulse (Figure 2-3), the peak of the reflected pulse occurs earlier and the latter half of the pulse has lower intensity compared to the reflected pulse in the non-ablative case (Figure 2-2). This drop in intensity for the ablative case represents a phenomenon that is referred to as a truncation effect.

Figure 2-2. Incident and reflected waveforms for a sub-ablative excimer laser pulse striking a glass target. Intensity is arbitrary.
Ablation rate is also considered to be a function of corneal hydration, as discussed in Section 1.7. This would suggest that varying hydration might also cause differences in truncation of the reflected waveform. As an illustrative example, experiments were done in which bovine eyes, after removal of the epithelium, were ablated at their normal hydration level and then after being exposed to a moderate flow of dry nitrogen gas for several minutes. Typical incident and reflected waveforms from these experiments are shown in Figure 2-4, where the “dry” case was a result of exposure to nitrogen flow for five minutes.

The reflected waveform in the “wet” case, as shown in Figure 2-4, is truncated more on the trailing edge and is shifted more leftward when compared to the “dry” reflected waveform. This truncation, however, may not equally affect significant portions of the overall waveform, making quantitative analysis difficult. Previous studies
have attempted to quantify the truncation effect using the ratio of the pulsewidth of the reflected waveform to that of the incident waveform (Ediger et al., 1993; Pettit et al., 1991; Pettit et al., 1993; Pettit et al., 1995). The success of this method was limited, however, by a lack of sensitivity. It was considered necessary, therefore, to find a more suitable method to quantify and perhaps even emphasize the truncation of the reflected waveform. The method of cross-correlation is a technique that was developed as part of the current work.

![Figure 2-4](image_url)

Figure 2-4. Typical waveforms for laser pulses during ablation of bovine corneal tissue under different hydration conditions. The waveforms have been scaled and shifted for alignment.

Many different forms of the autocorrelation function exist, and they have been applied in a variety of scientific fields, including combustion, astronomy, aerosol dynamics, and mathematics (Berne & Pecora, 1990; Dutilleul, 1995; Friedlander, 2000; Helmstedt & Schäfer, 1994; In ‘T Zand & Fenimore, 1996; Kirsch, Frenz, Schartl, Bartsch & Sillescu, 1996). Assuming a function $A(t)$ that varies with time, it is possible
to determine the value of the function at any time (t) or at any other point (t+τ) that is
delayed from the original time by a given value (τ). The two values, A(t) and A(t+τ), are
highly correlated as τ approaches zero, and are in fact identical, when the delay is equal
to zero (τ=0). As the delay is increased, the two values become less correlated until the
point where the delay exceeds the time domain of the function A(t) and there is zero
correlation between the two values. Typically, the autocorrelation function is described
as follows:

$$\langle A(0)A(\tau) \rangle = \lim_{\tau \to 0} \frac{1}{T} \int_{0}^{T} A(t)A(t+\tau)dt$$ \hspace{1cm} (2-1)

If the time domain is divided into discrete intervals, as is the case for a digitized
waveform, then it is possible to approximate the integral as a series and to then describe
the autocorrelation function as follows:

$$\langle A(0)A(\tau) \rangle \cong \lim_{N \to \infty} \frac{1}{N} \sum_{j=1}^{N} A_j A_{j+n}$$ \hspace{1cm} (2-2)

In Eq. 2-2, j and n are determined by t=jΔt and τ=nΔt, respectively, and this equation
becomes an increasingly accurate approximation to the integral in Eq. 2-1 as the time
interval Δt approaches zero (Berne & Pecora, 1990).

A cross-correlation function is similar in nature to an autocorrelation function, but
differs in that it shows the degree of correlation between two different functions which
can be labeled A(t) and B(t). The cross-correlation function between these two can be
described as follows:

$$\langle A(0)B(\tau) \rangle \cong \lim_{N \to \infty} \frac{1}{N} \sum_{j=1}^{N} A_j B_{j+n}$$ \hspace{1cm} (2-3)
The nature of the cross-correlation function is not as clear as that of the autocorrelation function, because there are now two different functions whose behavior may be completely independent of each other. Finally, a correlation function varies, and can be plotted, as a function of the time delay $\tau$. For two functions with finite time domain, the correlation function will ultimately reach zero.

To eliminate the issue of scale and make the cross-correlation a function of the waveform shape only, the reflected signal was always normalized to the incident signal. Therefore, the peak values of the two waveforms were always the same. To compensate for the pathlength difference between the measured incident and reflected signals, the reflected waveform for each laser pulse was shifted forward in time the appropriate amount, as determined from sub-ablative laser pulse measurements on a glass slide. This was done to ensure that the correlation function was dependent on the true relationship between the incident and reflected waveform shapes and not simply an artifact of the pathlength difference. The best resolution of the oscilloscope was 0.4 ns, meaning that the waveforms were digitized into discrete data points that were spaced 0.4 ns from each other. This 0.4-ns time step was chosen as the value of $\Delta t$ for the discrete cross-correlation function. For a given pair of incident and reflected waveforms, the cross-correlation function for each ablation site was evaluated using the following process:

1. For $\tau=0$, the intensity values of the incident and reflected waveforms at each time step were multiplied together, and these values were then summed.

2. For $\tau=0.4$ ns, the reflected waveform was shifted one step later in time and the intensity values were then multiplied together. Therefore, the first reflected data point was multiplied by the second incident data point, the second reflected data point by the third incident point, and so on. The products were then summed. This process was continued for each delay time, with the reflected waveform being shifted by an increasing number of steps.
3. Once the delay exceeded the width (in time) of the incident waveform, the process was terminated. Thus a raw cross-correlation function was defined as a function of time delay. The correlation function was then normalized by dividing each value by the autocorrelation of the incident waveform evaluated for zero time delay. This normalization factor was calculated by squaring each data point of the incident waveform and summing these values, and was used to eliminate the effect of absolute signal, thereby mitigating any effects of signal fluctuations from pulse to pulse, site to site, or day to day.

The general form of a cross-correlation function, evaluated for discrete intervals, was given in Eq. 2-3. For the purposes of the current work, the specific form of the cross-correlation function, evaluated according to steps 1-3 above, is given by:

$$
\langle R(0)I(\tau) \rangle = \frac{1}{\langle I(0)I(0) \rangle} \sum_{j=1}^{N} R_{j}I_{j+n}
$$

(2-4)

In Eq. 2-4, the normalization factor, which is the autocorrelation function of the incident waveform for zero time delay (i.e., \(\tau=0\)), is shown immediately before the series.

The process of evaluating the cross-correlation function is illustrated in Figure 2-5. For each value of \(\tau\), the calculation begins at the first point of the reflected waveform, and continues until the point where the incident waveform is no longer defined. Beyond this point in time, the product of the two waveforms is defined as zero and no longer contributes to the summation. Eventually, the time delay becomes so large that the entire reflected waveform is beyond the point where the incident waveform is defined. At this point, the cross-correlation function is identically zero and the calculation is terminated. Figure 2-5 shows an incident waveform, which remains fixed, and a reflected waveform at delays of 0, 8, and 20 ns. Although it appears that the reflected waveform is being increasingly shifted later in time, it is mathematically equivalent to shift the incident waveform increasingly forward in time.
Figure 2-5. Example waveforms illustrating the method of cross-correlation. The incident (dashed) waveform remains fixed while the reflected (solid) waveform is shifted in time by the amount indicated by the value of $\tau$.

Shown in Figure 2-6 are examples of cross-correlation functions derived from bovine eye data taken for varying laser pulse energy. One of the functions was derived from waveforms corresponding to ablation at approximately 2.8 mJ/pulse and the other was derived from waveforms corresponding to ablation at approximately 4.5 mJ/pulse. An obvious difference between the two functions is the different rates of decay occurring after the peak values. Figure 2-7 is a closer view centering on the approximately linear decay portion of the same correlation functions. Closer inspection of the initially rising portion of the correlation functions, before the peak, revealed a less obvious difference. Although it is somewhat difficult to see, the initial slopes of the two functions are actually different. To illustrate this point, a closer view centering on the initial portion of the correlation functions is shown in Figure 2-8.
Figure 2-6. Cross-correlation functions as a function of time delay ($\tau$) corresponding to ablation of bovine corneal tissue using two different laser pulse energy levels.

Figure 2-7. Closer view of the linear decay portions of the cross-correlation functions shown in Figure 2-6, illustrating the difference between the decay rates.
Figure 2-8. Closer view of the initially rising portions of the cross-correlation functions shown in Figure 2-6, illustrating the difference between the initial slopes.

Quantifying the difference between the decay rates was accomplished by fitting a line, using linear least-squares regression, to the data points spanning from 3.2 ns to 8.4 ns, inclusive of the two end points. The slope of this line, which is clearly negative, represents the decay rate of the function. For the cross-correlation functions shown in Figures 2-6, 2-7, and 2-8, the decay slopes (ignoring the negative sign) are 0.064 and 0.040 at 2.8 and 4.5 mJ/pulse, respectively.

Quantifying the difference between the initial slopes was done in a similar manner. The instantaneous slope at the origin (\(\tau=0\)) was used to accomplish this. Initially, the procedure for calculating the slope was to fit a quadratic to the first four data points using least-squares regression and to differentiate the quadratic function. This method was sufficient, but somewhat computationally intensive, so the process was altered to use straightforward numerical methods. Assuming a function \(f(x)\), which in this case would
be the cross-correlation function, a standard numerical technique to calculate the slope at any point \( x \) is to apply a forward finite-divided difference formula:

\[
\begin{align*}
  f'(x) &= \frac{2f(x+3h) - 9f(x+2h) + 18f(x+h) - 11f(x)}{6h} \\
  &\quad \text{(2-5)}
\end{align*}
\]

In Eq. 2-5, the slope is \( f'(x) \) and \( h \) is the incremental step, which in this case is \( \Delta t \). As applied, this formula provides a value with accuracy of order \( h^2 \). Therefore, the slope at the origin was calculated using Eq. 2-5, where \( x \) is zero, \( h \) is 0.4 ns, and \( f'(0) \) is the value of interest. This was a more suitable method of calculating the slope because it is readily implemented and therefore suitable for real-time application, and it produced results that were typically within 1-2% of the value produced from the quadratic fit. For the cross-correlation functions shown in Figures 2-6, 2-7, and 2-8, the initial slopes are 0.097 and 0.083 at 2.8 and 4.5 mJ/pulse, respectively.

### 2.2 Confocal Raman Spectroscopy

A significant source of error may be introduced in refractive procedures if an individual patient’s cornea has an ablation rate that varies appreciably from the average clinical rate. As discussed previously, the corneal ablation rate has been linked to the state of corneal hydration, which makes an understanding of the transient nature of corneal hydration for clinically relevant conditions an important issue. This section details the use of a confocal Raman system for the assessment of bovine corneal hydration as a function of time corresponding to different surface treatment methodologies. This study, and all subsequent studies using animal tissues, was done in accordance with the University Institutional Research Board policies regarding the use of animals. More information concerning these policies can be found at [http://acs.ufl.edu/](http://acs.ufl.edu/).
2.2.1 Experimental Equipment and Procedures

Whole bovine eye globes were collected within 30 minutes of animal sacrifice and stored in phosphate-buffered saline at ambient temperature (~22°C) prior to experiments. All Raman measurements were made between two and four hours post mortem. Eyes were collected and stored in pairs for each bovine, and subsequently used in pairs for the Raman measurements as discussed below. After performing Raman spectroscopy, select bovine cornea were harvested and measured at the cornea center using a precision linear caliper (12.5-µm accuracy). The bovine corneas were found to have an average full thickness of 750 ± 50 µm (compared with average human central corneal thickness of 520 µm).

A confocal micro-Raman spectrometer system, listed in Table 2-1, was used for all measurements. Figure 2-9 shows a schematic diagram of the Raman system, including the main excitation and collection components. The excitation source was a 632.8-nm helium-neon laser operating with nominal output energy of 15 mW. The laser beam was focused on the sample using a 10x objective resulting in a focused beam spot of 35 µm on the corneal sample surface. The laser beam was attenuated with a neutral density filter prior to entering the microscope objective to yield a laser beam power of 2.8 mW on the sample surface. It was determined through experimental repeatability and examination using visible microscopy that no effects on the corneal tissue were induced by the laser beam intensity under these conditions.
Raman scattered light was collected in the backscatter mode through the microscope objective and dispersed onto a 1024-pixel CCD detector array using a 600 grooves/mm grating. The grating was centered to provide a spectral window between 735 and 862 nm over the CCD array, corresponding to a Raman shift ranging from 2215 to 4205 cm\(^{-1}\). The effective spectral dispersion was 0.12 nm/pixel, equal to about 1.6 to 2.3 cm\(^{-1}\)/pixel over the specified wavelength range. The Raman microscope was configured for confocal microscopy, with the confocal aperture set to a diameter of 500 µm. This resulted in an effective integration depth of 300 µm, thereby ensuring a volume-integrated measurement of corneal tissue rather than a surface-weighted measurement. All Raman spectra were recorded using a single acquisition time (no spectral averaging) of ten seconds for the corneas and for the water/acetone solutions.

To assess the signal linearity of the Raman technique as a relative measure of hydration, known solutions of acetone and deionized water were prepared and analyzed. All solutions were prepared using ultrapurified, deionized water (Fisher Scientific...
product W2-20) and electronic grade acetone (Fisher Scientific product A946-4). To eliminate changes in solution concentration due to preferential acetone evaporation, all solutions were prepared and used immediately.

Next, thirty attached bovine corneas (whole globes) were equally divided into three experimental groups. The first group (ten corneas) was designated the control group, and the intact corneas were used as collected and stored. The second group (ten corneas) consisted of eyes that were de-epithelialized using a scalpel edge to create a 10-12 mm optical zone (OZ). Clinically, removal of the epithelium is called manual debridement; therefore, this group was designated the MD group. The third group (ten corneas) was designated the lamellar keratectomy (LK) group, as corneal lamellar flaps were manually cut using a scalpel and removed from the surface. The total flap thickness of three different flaps ranged from 100 to 250 µm, as directly measured with the precision calipers. Each of the three experimental groups (control, MD, and LK) was comprised of ten corneas from five bovines.

Each group was further divided into two subgroups of five corneas. The corneas were paired for this division; therefore each subgroup received a single eye from each of the five bovines. The first subgroup was exposed to quiescent ambient air for a period of six minutes, with a Raman spectrum recorded every thirty seconds. The second subgroup was exposed to a forced flow of compressed nitrogen tangential to the cornea surface, with a Raman spectrum recorded every thirty seconds for six minutes. The forced nitrogen flow was achieved using a flow rate of 9 liters/min through a 3.5-mm inner diameter stainless steel tube. The tube was positioned about 3 cm from the cornea with the nitrogen flow directed tangentially across the cornea surface. The mean flow velocity
at a distance of 12 mm from the exit of the tube was 20 m/s, and the mean velocity directly above the corneal surface was about 7 to 11 m/s, as measured using a hot-wire velocity probe. The diameter-based Reynolds number (gas density*velocity*tube diameter/gas viscosity) of the nitrogen flow was about 1000, which corresponded to a laminar flow of nitrogen.

For the corneas exposed to the nitrogen flow, an initial Raman spectrum was recorded prior to initiation of the flow. With this experimental matrix, changes in corneal hydration due to drying under either ambient or forced-flow conditions were assessed using paired groups (N=5) for the control, MD, and LK treatments.

2.2.2 Clinical Relevance

While this study is designed to assess transient changes in bovine corneal hydration, it is useful to relate the current experimental conditions to those corresponding to clinical excimer laser refractive procedures. A clinical excimer laser system uses vacuum suction to draw air across the surface of the cornea, which causes aspiration of the ablation plume. To provide a comparison with clinical systems, velocity measurements were recorded within the flow field induced above a human subject with a clinical LASIK system (VISX Star S3) under representative clinical conditions. The aspiration tube (22 mm diameter) was positioned about 3 to 4 cm from the human cornea and pointed toward the corneal surface such that the axial centerline of the tube was about 3 cm above the corneal surface. The air velocity induced by the aspirator near the tube inlet was 20 m/s, and the velocity of the induced flow field above the cornea was about 1 to 1.3 m/s.

While the clinical aspirator entrance velocity and the current nitrogen jet exit velocity were identical, the flow velocity above the corneal surface was markedly greater
for the nitrogen jet case. This increased velocity reflects the relative confinement of the nitrogen jet momentum (i.e., flow field) to the centerline of the exit nozzle. In contrast, the clinical aspirator draws in air equally from all directions resulting in a more diffuse and lessened flow field several centimeters from the entrance.

It is noted that the micro-Raman measurements were recorded in a sealed (light tight) sample chamber, which precludes the direct emulation of the open-air flow patterns created by clinical system aspirators. Furthermore, no active control of ambient air humidity was available in the present study. It is noted that several research groups have reported the effects of or need to control humidity during LASIK procedures (Doane, Koppes & Slade, 1996; De Souza et al., 2001; Krueger, Campos, Wang, Lee & McDonnell, 1993; Walter & Stevenson, 2004).

2.3 Ablation Rate Measurements Using White-Light Interferometry

Despite the relatively high degree of accuracy and precision of clinical LASIK and PRK systems in reshaping the cornea, one important limitation remains, namely that the amount of corneal tissue removed per laser shot, known as the ablation rate, may differ from patient to patient, between eyes for a given patient, and perhaps even over the course of treatment for a given eye. In clinical laser systems, unfortunately, no means currently exist for real-time correction to refractive treatment algorithms.

In view of these issues, efforts to further enhance the precision of laser refractive procedures can benefit from a better understanding of the corneal ablation process. Such research efforts can benefit from a means to precisely measure ablation rates. This section presents a novel method of measuring the ablation rate of corneal tissue using wax impressions of ablation craters in combination with a scanning white-light
interferometer. Additional data are presented for PMMA ablation to support the current approach. The experimental configuration is shown in Figure 2-10.

Figure 2-10. Experimental configuration for bovine eye ablation rate measurements.

Ablation was performed using the Alcon ArF excimer laser listed in Table 2-1. The laser pulse energy was maintained at a nominal value of 2.7 mJ/pulse and the pulse repetition rate was set between 2 and 4 Hz. A shutter, also listed in Table 2-1, was used to control and count the delivery of laser pulses. A low-power 532-nm diode laser was aligned co-axially with the excimer laser beam to ensure that the incident laser beam was always normal to the target surface. A low-power 670-nm diode laser beam was aimed tangentially across the target surface and functioned to align the target surface at a constant plane with respect to the excimer laser focal point. In other words, the diode
laser was used to ensure that the target was at the focal point of the laser. Table 2-3 contains a listing of equipment used for the ablation rate measurements.

Table 2-3. Listing of equipment for bovine eye ablation rate experiments.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>532-nm alignment laser</td>
<td>532-nm diode-pumped solid state laser, Max 5 mW</td>
<td>Intelite</td>
</tr>
<tr>
<td>670-nm alignment laser</td>
<td>670-nm diode laser, Max 3 mW</td>
<td>Imatronic</td>
</tr>
<tr>
<td>Wax melter kit</td>
<td>Heated palette and aluminum cups</td>
<td>Dick Blick Art Materials (64902-1009)</td>
</tr>
<tr>
<td>Wax pen</td>
<td>Squeezable aluminum wax pen (package of 10)</td>
<td>Dick Blick Art Materials (64902-1000)</td>
</tr>
</tbody>
</table>

The area of laser exposure was determined by ablating through black ink on a glass microscope slide, and measuring the resulting spot after several laser pulses. The average laser fluence over the exposure area was determined to be approximately 350 mJ/cm$^2$.

The laser beam profile is nominally “bullet” shaped (i.e., truncated Gaussian), hence the peak fluence for the current study was about 600 mJ/cm$^2$ in the central portion of the beam. This is somewhat higher than the often-reported typical clinical fluence of 130-250 mJ/cm$^2$ for large-beam systems, but is consistent with the clinical fluence of the Alcon laser beam system.

2.3.1 PMMA Ablation Experiments

In the first set of experiments, a 6-mm thick block of black PMMA (Plexiglas) was ablated with the excimer laser beam. The configuration was the same as shown in Figure 2-10, but with the PMMA block as the target instead of a bovine eye. The ablation was carried out in a pattern of three rows each with four separate areas of exposure. The four exposure areas in each row were treated with different numbers of laser pulses, specifically 14, 21, 28, and 35 pulses. The laser pulse energy was confirmed before the
ablation of each row. Immediately following ablation all twelve craters, which were facing upward, were covered with liquid paraffin wax using an aluminum wax pen, which resembled a medicine dropper. The wax was allowed to cool and solidify as a single unit for approximately 15 minutes before being carefully removed with fine tweezers. It is noted that the wax was observed to solidify in a few seconds, and essentially reached its original hardness in about one to two minutes.

Both the original PMMA ablation craters and the corresponding wax impressions of the craters were examined using the white-light interferometer listed in Table 2-1. The interferometer was used in the vertical scanning mode, with an effective magnification of 5.2x, which produced a field of view measuring 1.2 mm by 0.9 mm. The depth of scan was adjusted in the range of 50-75 µm for each individual crater to ensure the presence of useful interference fringes across the entire field of view. A magnification of 5.2x enabled the entire ablation crater to be visible in the field of view. Ablation craters and corresponding wax impressions were scanned individually to maximize resolution and enhance the accuracy of depth measurements.

Each crater and each corresponding wax impression were scanned once and the results were stored as individual data sets. The system software provided a three-dimensional topographical image of the scanned surface, from which a two-dimensional depth profile can be constructed for any selected cross-section. To facilitate direct comparisons in the present study, the topographical images of the wax impressions (i.e., peaks) were inverted in the software to appear as craters, which were similar to the actual ablation crater images. For each PMMA crater and wax impression topographical image, a longitudinal cross-section was defined to best bisect the ablation crater through the
deepest portion. This process was repeated three times and the corresponding cross-sectional depth profiles were saved.

For each image, the three depth profiles were then averaged together to yield a single representative ablation depth profile. The resulting profiles were then plotted and analyzed. For a given cross-section, the ablation depth was determined using two different methods. The first, more rigorous method, referred to as Method 1, is shown in Figure 2-11 for a representative PMMA depth profile corresponding to 28 laser pulses.

![Figure 2-11](image)

Figure 2-11. Representative depth profile of a PMMA ablation crater corresponding to 28 laser pulses. Method 1, in which the crater floor is fit with a least-squares straight line, is illustrated for this depth profile.

A straight line, labeled “Line 1” in the figure, was drawn along the left side of the crater to approximate the section that intersects the crater floor. Another straight line, labeled “Line 2” in the figure, was drawn along the right side of the crater to approximate the section of that side that intersects the crater floor. The intersection of these two lines
with the crater floor determined the left and right boundaries of the data points used to fit a least-squares straight line through the crater floor. A third line, labeled “Line 3” in the figure, was drawn to connect the left and right “shelves” of the crater, which represent the normal height and contour of the pristine surface on either side of the exposed crater area. Line 3 was drawn such that it preserved the overall contour of the unablated areas and approximated as best as possible the normal surface that would exist if no ablation took place. The crater depth was calculated as the vertical distance between the midpoint of the straight-line fit of the crater floor and the corresponding point on “Line 3”.

The second method, referred to as Method 2, was less rigorous because the crater floor was not fit with a least-squares straight line. Instead, a straight line was drawn manually to approximate the crater floor. The crater depth, however, was still calculated as the vertical distance between the crater floor and “Line 3” at the midpoint of the floor. An ablation rate was calculated for each crater, both for the wax impression and for the PMMA crater, by dividing the total depth of the crater by the corresponding number of laser pulses used to create the crater. Furthermore, the ablation rate was calculated using the depths calculated using both of the above methods. The ablation rates for both methods were compared to assess any bias that Method 2 may have introduced. For the remainder of the PMMA ablation study, only Method 2 was used to determine ablation depths, as based on the findings discussed below.

The entire process of defining the cross-sections in triplicate, and determining the ablation depth and corresponding rate using Method 2 was done a second time to examine the repeatability of the procedure. Finally, two-sided, paired Student’s t-tests were used to assess the statistical significance of any differences between the ablation
depths and rates as determined using the actual PMMA craters and those determined using the wax impressions of the craters.

2.3.2 Bovine Cornea Ablation Experiments

A bovine eye study was performed in a similar fashion to that described above for PMMA. Seven whole bovine eye globes were collected within approximately one hour of animal sacrifice and stored in phosphate-buffered saline at ambient temperature (~22°C) prior to the ablation experiments.

All experiments were performed between two and eight hours post mortem. Immediately prior to ablation, each eye was mechanically de-epithelialized using a scalpel edge to remove the epithelium. For each sample eye, four different sites were treated, each with a different number of laser pulses, specifically 16, 24, 32, or 40 pulses. The laser beam was directed vertically downward and the targeted bovine eyes faced upward. This enabled the liquid wax to be dropped onto the corneal surface immediately following ablation without the need to move the eye in any way. After dispensing the liquid wax, the wax impression was allowed to solidify for about one minute and then removed from the surface of the eye and placed on a microscope slide. A second wax impression of the same crater was created and subsequently removed. The eye was then rotated to create a new ablation site that excluded any part of the eye that had been previously exposed to liquid wax, and the next ablation treatment was performed.

The laser energy was monitored before the ablation of each eye to maintain constant fluence. Each exposed eye was moistened by dabbing with a distilled water-soaked tissue (Kim-Wipe) immediately prior to each ablation treatment to maintain a relatively consistent degree of hydration. This procedure produced no excess water on the cornea surface, but functioned to qualitatively maintain a constant appearance of the
eyes consistent with their normal state. Confocal Raman hydration measurements were performed using the method described in Section 2.2 immediately prior to the ablation of the first site and immediately after the completion of ablation of the final site for each eye. This was done to assess any possible changes in corneal hydration.

In the bovine eye study, only the ablation crater wax impressions were investigated with the white-light interferometer as discussed above. The depth of scan for this study was extended to 100 µm to obtain useful interference fringes. The same analysis techniques used for the PMMA were applied in these experiments. Cross-sections were chosen from the topographical image in triplicate and averaged, and the ablation depth and rate were calculated using only Method 2 as described above for each ablation site based on the corresponding wax impression. To assess any differences between replicate wax impressions for the same crater, both the first and second wax impressions were analyzed for all of the 40-shot craters. For all other ablation sites, the first wax impression was used for the interferometry measurements unless there were incomplete data in the resulting interferometric images. In these cases, the second wax impression was used for subsequent analysis.

2.4 Coordinated Measurements of Time-Resolved Reflectance, Corneal Hydration and Ablation Rate

2.4.1 Experimental Procedure

All of the equipment used in these experiments is listed in Tables 2-1, 2-2, and 2-3 and the experimental configuration is a combination of those presented in Figures 2-1 and 2-10 coupled with the Raman spectrometer system shown in Figure 2-9. For these experiments, the previously described methods were combined in the following manner:

1. A bovine eye was de-epithelialized with a scalpel edge and immediately affixed in the sealed (light-tight) chamber of the confocal Raman system, after which a 20-
second Raman spectrum was recorded. This step was repeated four times at different sites on the eye to obtain four individual Raman spectra.

2. The eye was removed from the Raman chamber and affixed at the focal spot of the excimer laser, after which the exposed surface was dabbed with a distilled water-soaked tissue (Kim-Wipe). The eye was then ablated with the appropriate number of laser pulses.

3. Heated, liquefied paraffin wax was applied to the ablation zone using an aluminum dropper (i.e., wax pen) that was held approximately 5 mm above the ablated surface. The wax was allowed to cool and re-solidify for 1 minute and was then removed with a fine tweezer. This process was repeated such that at least two viable wax impressions were obtained.

4. The eye was rotated to expose a new ablation zone and the ablation process (i.e., steps 2 and 3) was repeated. A total of three sites were ablated.

5. After the final ablation was performed and the corresponding wax mold removed, the eye was removed from the ablation setup and once again affixed in the Raman chamber. Four 20-second Raman spectra, corresponding to four different sites, were recorded to assess the post-ablation hydration state of the cornea.

A total of eight bovine eyes were used for these coordinated experiments and the laser was operated at a repetition rate of 2 Hz. Six of the eyes were ablated with the same nominal laser energy of 3.4 mJ/pulse, although temporal fluctuations in the true laser energy were unavoidable. For these six eyes, three separate sites were ablated with 25 laser pulses at each site. One of the remaining bovine eyes was ablated with 25 laser pulses at each of three sites, with nominal laser energy of 4.1 mJ/pulse. The final eye was ablated, at nominal laser energy of 2.6 mJ/pulse, with 30 laser pulses at each of three sites. During the ablation of each site on each bovine eye, the incident and reflected waveforms corresponding to each ablating laser pulse were acquired by the oscilloscope and saved on a computer. The waveforms were later used to construct cross-correlation functions, and the initial and decay slopes of each correlation function were calculated as described in Section 2.1.2.
Since Raman hydration measurements could not be performed at the time of ablation, it was decided that the corneal hydration level could be determined for each ablation site by interpolation. Raman measurements were performed before ablation of the first site and after ablation of the final site for each eye, and the difference between these two hydration values was divided into equal intervals according to the number of sites ablated on each eye. This, of course, required the assumption of equal time intervals between ablation of each site. All sites were then assigned a hydration value based on the order in which they were ablated, with the first site having a hydration level closest to the pre-ablation condition and the final site having a hydration level closest to the post-ablation condition.

Once the experiments were completed and all bovine eyes had been ablated on a given day, the wax molds of the ablation craters were examined using a white-light interferometer. As described in Section 2.3, the interferometer was used to produce topographical maps of the craters, which were then used to calculate depths and ablation rates. Once this exercise was complete, all of the data, including the cross-correlation slopes, hydration measurements, and ablation rates, were consolidated for analysis.

2.4.2 Energy Calibration for the Alcon ArF Excimer Laser

The incident waveforms collected for each laser pulse served another purpose in addition to their use in constructing cross-correlation functions. The integrated peak area of the incident waveform was verified as proportional to the laser energy. Therefore, the laser pulse energy during ablation of each individual site was quantified by calibrating the integrated peak of the incident laser beam waveform against the measured laser energy, a process that was performed at the outset of experimentation on a given day to account for daily fluctuations in the instrumentation and the laser itself.
To calibrate the laser energy, 200-pulse average waveforms corresponding to the incident laser beam were recorded in triplicate at three different laser energy levels. While each average waveform was being recorded, the laser power was observed using an integrating calorimeter connected to a power meter, both of which are described in Table 2-4. Since the calorimeter and power meter provided a measurement of the laser power (energy per unit time), it was necessary to divide by the pulse repetition rate to determine the actual laser pulse energy. For calibration measurements, the laser was operated at 50 Hz, and waveforms were recorded in triplicate for laser power levels of 130, 180, and 230 milliWatts (mW), corresponding to laser pulse energy levels of approximately 2.4, 3.4, and 4.3 mJ, respectively. Figure 2-12 shows three 200-pulse average incident waveforms corresponding to these three laser energy levels.

It is clear that there is a monotonic increase in the integrated area of the waveform with increasing energy. Figure 2-13 shows the calculated incident peak area as a function of the laser pulse energy, including information from the waveforms in Figure 2-12 as well as from the other two measurements at each energy level. Clearly the relationship between the peak area of the incident laser pulse and the laser energy is highly linear and the measurements include only slight experimental error, as illustrated by the small error bars. It was concluded, therefore, that the integrated area of the incident laser pulse waveform can be used to determine the laser pulse energy with a great deal of accuracy.

Table 2-4. Listing of equipment for Alcon laser energy calibration.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Manufacturer (Model or Part #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorimeter</td>
<td>Volume absorbing calorimeter, 4.75” diameter, Max 50 W/cm², Max 50-mm beam diameter, 190-360 nm spectral response</td>
<td>Scientech (Astral AC50UV)</td>
</tr>
<tr>
<td>Power meter</td>
<td>Power meter for use with calorimeter, Min range 3.0 mW, Max range 30 W</td>
<td>Scientech (Vector S310)</td>
</tr>
</tbody>
</table>
Figure 2-12. Incident waveforms corresponding to the averages of 200 laser beam pulses for three different laser energy levels of 2.4, 3.4, and 4.3 mJ/pulse.

Figure 2-13. Integrated peak area of incident waveforms as a function of measured laser pulse energy. Each data point is the average of three \((N=3)\) peak area measurements, each of which corresponds to a waveform that is the average of 200 incident laser pulses. The error bars represent ± one standard deviation and the solid line represents the least-squares regression line.
2.5 Transmission Experiments

Some research has been done to examine the transmission of 193-nm excimer laser light through various materials relevant to excimer laser corneal ablation, including corneal tissue, collagen, human tears, and salt solutions (Dair et al., 2001; Ediger et al., 1993a; Ediger, Pettit & Hahn, 1994; Michalos et al., 1994; Pettit & Ediger, 1993, 1996). The aim of these studies was to determine the optical properties of the individual constituents of corneal tissue with respect to 193-nm laser light.

For the current research, there were three major types of materials through which 193-nm laser light transmission was measured—water, aqueous solutions of collagen and amino acids, and thin collagen films. All of the major equipment used in these experiments can be found in Tables 2-1 and 2-2, and the remaining items are included in the discussion below.

2.5.1 Water Film Transmission Measurements

Figure 2-14 shows the collection geometry and instrumentation, including the Alcon ArF excimer laser, for the water film transmission experiments. The equipment, including the laser, optics, and instrumentation, used in these experiments can be found in Tables 2-1 and 2-2. The two types of water for which transmission was measured and the quartz flat on which the water films were created are detailed in Table 2-5.

Table 2-5. Listing of equipment for water film transmission experiments.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Manufacturer (Model or Part #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>Deionized ultra-filtered water, 20-L bottle</td>
<td>Fisher Scientific (W2-20)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>Zephyrhills distilled water</td>
<td>Zephyrhills</td>
</tr>
<tr>
<td>Cuvette</td>
<td>UV-grade cuvette, Suprasil quartz, 10-mm pathlength, 3.0-mL capacity</td>
<td>Fisher Scientific (14-385-918A)</td>
</tr>
<tr>
<td>Quartz flat</td>
<td>UV-excimer window, Suprasil 312, 2.0” diameter, 0.187” thickness</td>
<td>Heraeus Amersil (31285)</td>
</tr>
</tbody>
</table>
Figure 2-14. Configuration for water film transmission experiments.

First, the transmission was measured for deionized water in a UV-grade cuvette at an extremely low, non-ablative laser fluence to determine the “small-signal” (i.e., low laser fluence) absorption coefficient of deionized water. Transmission and the absorption coefficient are related by the Beer-Lambert law, which was presented in Eq. 1-1 and is shown below:

\[ \tau = e^{-\alpha L} \]  

(2-6)

In this equation, \( \tau \) is the measured transmission, \( L \) is the pathlength (cm), and \( \alpha \) is the absorption coefficient (cm\(^{-1}\)). The relationship can be inverted to determine the absorption coefficient from a measured transmission through a known pathlength.

The ratio of the integrated transmission signal to the integrated incident signal was measured three times through the empty cuvette, then again three times through the cuvette containing deionized water. The ratios measured through the empty cuvette were
averaged together, and the same was done for the ratios measured through the full cuvette. Transmission was determined by dividing the average ratio through the water-filled cuvette by the average ratio through the empty cuvette. However, it was also necessary to correct for the Fresnel reflection effects due to refractive index differences at the two water-cuvette interfaces. A formulation for the refractive index of water is presented in Appendix A, while Fresnel effects are detailed in Appendix B.

The transmission of both deionized and distilled water was then measured as a function of laser energy. First, the ratio of the transmitted to incident signal was measured through a quartz flat for ten laser energy levels ranging from approximately 0.9 to 3.8 mJ/pulse. These measurements were done in triplicate. The same was then done for a quartz flat with an appropriate water (deionized or distilled) film deposited on its top (i.e., horizontal) surface. The corresponding ratios were then calculated to determine the transmission of the water itself, again correcting for Fresnel effects as explained in Appendix B.

It was assumed that the absorption coefficient at the lowest laser energy level was equal to the small-signal value. Therefore, it was possible to use Eq. 2-6 with the measured transmission at this laser energy and the known small-signal value of the absorption coefficient to determine an effective film thickness. Assuming a constant film thickness throughout the experiments, the absorption coefficient of water (both deionized and distilled) was determined as a function of laser energy. As mentioned in Section 2.4.1, the laser energy naturally fluctuates somewhat over time. Therefore, the individual energy values at a given level, as calculated using the calibration procedure outlined in Section 2.4.2, were averaged together and assigned a standard deviation.
In addition, transmission was measured through quiescent air as a function of laser energy to determine if any trends in the quartz flat transmission were due to the quartz itself or due to the surroundings. As before, transmission measurements were done in triplicate for laser energy levels ranging from approximately 0.9 to 3.8 mJ/pulse. All measurements performed for air, quartz, deionized water, and distilled water were done by ensemble averaging the signals from 500 laser pulses. Since these were not ablation experiments, single-shot transmission was not considered relevant.

2.5.2 Transmission Through Solutions of Collagen and Amino Acids

The experimental setup and collection geometry for these transmission experiments is shown in Figure 2-15. The transmission cell was constructed by pressing two 50 mm diameter quartz flats together with a thin o-ring between them that served to contain the solution of interest. The components of the transmission cell, including the chemical compounds used to create the solutions, are detailed in Table 2-6.

![Figure 2-15. Experimental configuration and optical collection geometry for transmission measurements through solutions of collagen and amino acids.](image)
Table 2-6. Components of transmission cell and chemical compounds for solutions.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz flat</td>
<td>UV-excimer window, Suprasil 312, 2.0” diameter, 0.187” thickness</td>
<td>Heraeus Amersil (31285)</td>
</tr>
<tr>
<td>O-ring</td>
<td>~0.063” thickness, ~1.35” outer diameter</td>
<td>N/A</td>
</tr>
<tr>
<td>Deionized water</td>
<td>Deionized ultra-filtered water, 20-L bottle</td>
<td>Fisher Scientific (W2-20)</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.5 Normal (N) acetic acid, Diluted from 1.0 N stock solution</td>
<td>Sigma Chemical (31859-0)</td>
</tr>
<tr>
<td>Collagen</td>
<td>Solid type III calfskin collagen, Acid soluble</td>
<td>Sigma Chemical (C-3511)</td>
</tr>
<tr>
<td>Glycine</td>
<td>SigmaUltra &gt; 99% (titration), Solid powder</td>
<td>Sigma Chemical (G7403)</td>
</tr>
<tr>
<td>L-proline</td>
<td>BioChemika Ultra ≥ 99.5% (NT), Solid powder</td>
<td>Fluka (81709)</td>
</tr>
<tr>
<td>Cis-4-hydroxy-L-proline</td>
<td>Puriss. ≥ 99.0% (NT), Solid powder</td>
<td>Fluka (56248)</td>
</tr>
</tbody>
</table>

The major equipment, including the laser, optics, and detection instrumentation, are all detailed in Tables 2-1 and 2-2. The laser used in these experiments was the Alcon ArF excimer laser. Transmission of 193-nm ArF excimer laser light at a sub-ablative fluence of approximately 4 mJ/cm² was measured through four solutions, namely water, acetic acid, dissolved collagen in acetic acid, and dissolved amino acids in acetic acid. The exact optical pathlength of the transmission cell was determined by the difference between the measured thickness of the cell and the measured total thickness of the two glass flats alone (i.e., no o-ring). These measurements were made using a precision micrometer with an uncertainty of ± 12 µm, and the average pathlength of the transmission cell was measured as 0.167 cm. For all experiments, 193-nm laser line filters were used in front of the photodectors to eliminate any spurious signal due to fluorescence, which was noted to be significant if the filters were removed.
For each solution transmission measurement, an average laser waveform was recorded first without the cell present, then through the cell containing the appropriate solution, then again without the cell present. Neutral density filters were used as necessary to maintain signal linearity over a finite range. Transmission was calculated as the ratio of the integrated waveform (i.e., full peak area) recorded through the cell to the average of the two integrated waveforms recorded without the cell present, correcting as necessary for any differences in optical density due to the use of neutral density filters.

Pure deionized water was used as a control to verify repeatability, as well as to assess the validity of the measurements based on the widely reported absorption coefficient of water at 193 nm. Collagen solutions were created by dissolving appropriate amounts of solid type III calf skin collagen in 0.5 N acetic acid solution. A stock solution was created using 32 mg of collagen and 100 mL of acetic acid solution, and the resulting solution was stirred for 48 hours.

After 48 hours, transmission measurements were performed using the experimental procedure outlined below. A laser pulse waveform, the average of 200 individual laser pulses, was recorded without the cell present. The cell containing deionized water was then placed in the beam path, and a 200-pulse average waveform was recorded. The cell was removed, and a third 200-pulse average waveform was recorded. This process was repeated, but the middle waveform was recorded with the pure 0.5 N acetic acid solution in the cell. The process was repeated a third time, but with the middle waveform recorded with the desired solution strength of dissolved collagen in the cell. The collagen solution measurements were repeated in triplicate for each specified concentration, however, the acetic acid and deionized water measurements were recorded only once for
each set of measurements. The process was repeated for serially diluted collagen solutions over the desired range, namely 0.24, 0.18, 0.12, and 0.06 mg/mL.

To assess the degree to which the peptide bond is the primary chromophore for 193-nm radiation, additional transmission measurements were made using equivalent concentrations of isolated amino acids (i.e., containing no peptide bonds). These amino acid solutions were created by dissolving appropriate amounts of solid powders of glycine, L-proline, and cis-4-Hydroxy-L-proline in 0.5 N acetic acid solution. Specifically, a stock solution of 5 mg/mL was created using nominally equal amounts (100 mg each) of glycine, proline, and hydroxyproline (300 mg of total amino acids) dissolved into 60 mL of acetic acid solution. The resulting solution was then stirred for 48 hours. The stock amino acid solution was then diluted to produce final concentrations equal to 1.25, 1.0, and 0.75 mg/mL. For each solution concentration, transmission measurements were recorded in the same manner as for the collagen solutions, including the deionized water and acetic acid reference measurements.

The absorption coefficient of deionized water, as measured in these experiments, was compared to the small-signal measurements performed in the cuvette (detailed in Section 2.5.1). Given that absorption coefficients are additive, the absorption coefficients of the acetic acid and collagen solutions were calculated directly from the Beer-Lambert law as follows:

\[
\alpha_{\text{acid}} = \alpha_{\text{water}} - \frac{\ln \left( \frac{\tau_{\text{acid}}}{\tau_{\text{water}}} \right)}{L} \tag{2-7}
\]

\[
\alpha_{\text{collagen}} = \alpha_{\text{water}} - \alpha_{\text{acid}} - \frac{\ln \left( \frac{\tau_{\text{solution}}}{\tau_{\text{water}}} \right)}{L} \tag{2-8}
\]
In determining the absorption coefficient of water, it was necessary to account for Fresnel losses to calculate an absolute value. However, Fresnel losses were considered to be identical for all different solutions, and were therefore neglected when calculating the absorption coefficients of acetic acid and collagen due to the ratio of transmission values in Eqs. 2-7 and 2-8.

In determining the absorption coefficient of collagen, the average values of $\tau_{\text{water}}$, $\alpha_{\text{water}}$, and $\alpha_{\text{acid}}$, as measured over multiple experiments, were used to reduce the collagen solution data. Because each collagen solution measurement yielded a corresponding absorption coefficient, it was necessary to normalize the data by the actual collagen bond density. The following relation was used to calculate the equivalent peptide bond number density, N (in bonds/cm$^3$), for each collagen solution:

$$N = \left( X \cdot \frac{g}{cm^3} \right) \left( \frac{3465 \text{ bonds}}{\text{macromolecule}} \right) \left( \frac{6.022 \times 10^{23} \text{ macromolecules}}{\text{mole}} \right) \left( \frac{308,300 \text{ g}}{\text{mole}} \right)$$

(2-9)

In Eq. 2-9, $X$ is the given collagen solution mass concentration and the other quantities were explained and derived in Section 1.1.3. Eq. 2-9 enables correlation of the measured absorption coefficient of the dissolved collagen solutions as a function of the peptide bond number density (i.e., chromophore density). The results of the transmission measurements of the 1:1:1 glycine, proline, and hydroxyproline solutions were compared to those of the collagen solutions on a mass basis to determine the contribution of the amino acids to the overall absorption of 193-nm light in collagen. In other words, the absorption coefficients of the two types of solutions (i.e., collagen and amino acids) were plotted as a function of mass concentration and compared.
2.5.3 Thin Collagen Film Transmission Measurements

The experimental configuration and collection geometry for these measurements were exactly the same as for the water film transmission measurements, as shown in Figure 2-14. The only difference was that a thin collagen film on a quartz flat was used in place of a water film on a quartz flat. As with the dissolved collagen solution studies described in Section 2.5.2, type III calfskin collagen was dissolved in 0.5 N acetic acid to create a solution with nominal concentration of 1 mg/mL. The collagen solution was stirred for approximately 48 hours to completely dissolve all of the collagen.

Once dissolved, approximately 5 mL of solution was deposited on each of three individual 50-mm diameter quartz flats and allowed to dry for 48 hours. Once dry, each resulting collagen film was ablated using the same pattern of four rows with eight ablation sites in each row, for a total of 32 sites. The first and third rows were ablated from left to right starting at the lowest pulse energy value (approximately 0.9 mJ/pulse) and increasing the energy incrementally at each site to the highest pulse energy value (approximately 3.8 mJ/pulse). The second and fourth rows were ablated from left to right starting at the highest pulse energy and ending at the lowest energy. The ablation pattern, with respect to energy, was alternated between rows in an attempt to compensate for any variations in film thickness, and is illustrated in Figure 2-16.

At each ablation site, the collagen film was subjected to 25 laser pulses, which was sufficient to completely perforate the film for all pulse energies, enabling the last few pulses of the sequence to be used for normalization of the transmission values. For each ablation site, the pulse-to-pulse transmission was calculated based on signal normalization of the transmitted integrated pulse area divided by the integrated pulse area averaged over the final five laser pulses. As mentioned above, the final five pulses were
always found to correspond to essentially complete removal of the collagen film, hence they represent the reference transmission value (i.e., no film). This procedure yielded a pulse-to-pulse transmission value between zero and one for the first twenty laser pulses. For each laser pulse energy investigated, the transmission values for each of twelve ablation sites (one crater per energy level on each of four rows per film over a total of three films) were averaged together as a function of laser pulse number.

Figure 2-16. Ablation pattern for thin collagen film transmission experiments. The pattern from the lowest laser energy to the highest laser energy was alternated between rows. There were a total of eight ablation sites per row and four rows per film.

After ablating the films as described above, the edges of individual craters were scanned with a white-light interferometer at five distinct locations on each of the three films. The interferometer produced a two-dimensional profile showing the top surface of the unablated collagen film and the bottom surface of the ablated crater. It is noted that the bottom surface was actually the surface of the quartz flat since the films were fully ablated (i.e., completely removed) at the crater bottom. From the resulting interferometer
maps, the average thickness of the collagen films was determined using the fifteen individual measurements (five sites on each of three films).

The pulse-to-pulse progression of the transmission data was then used to calculate an equivalent ablation rate for each recorded laser pulse energy. Specifically, for each laser pulse energy, the ablation rate was calculated as the average film thickness divided by the number of laser pulses required to reach 50% normalized transmission.

2.6 Emission Experiments

The ablation process itself is not as of yet well understood or characterized. It has been suggested that transient species created by the excimer laser pulse may play a significant role in the ablation process, perhaps by acting as chromophores for the 193-nm laser light. Alternatively, the excimer laser pulse might initiate a laser-induced breakdown or plasma, resulting in significant transient absorption. This section presents emission experiments in which the primary goal was to provide evidence either supporting or refuting the possibility of a laser-induced plasma during the ablation process.

2.6.1 Ablation Emission Measurements

The major equipment, including the laser and collection instrumentation, used for the emission measurements are included in Table 2-1. A schematic of the experimental configuration for these experiments is shown in Figure 2-17. The ArF excimer laser made by GAM Laser, Inc. was used because of its high precision timing (i.e., low jitter), which is necessary due to the time-sensitive nature of these experiments. Additional optical equipment used for these experiments is listed in Table 2-7.
Figure 2-17. Experimental configuration and optical collection geometry for emission measurements.

Table 2-7. Optical components used for emission measurements.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Manufacturer</th>
<th>(Model or Part #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser focusing lens</td>
<td>Plano-convex lens, 50.8-mm diameter, 100-mm focal length, UV-grade fused silica</td>
<td>CVI Laser Corp.</td>
<td>(PLCX-50.8-51.5-UV)</td>
</tr>
<tr>
<td>Pierced mirror</td>
<td>High UV reflection (Alflex “A”) first surface mirror, 100 x 100 x 1 mm, 0.5” diameter hole through center</td>
<td>Rolyn Optics</td>
<td>(60.2475)</td>
</tr>
<tr>
<td>Collimating lens</td>
<td>Plano-convex lens, BK7 glass, 100-mm diameter, 160-mm focal length,</td>
<td>Comar Optics</td>
<td>(160-PQ-100)</td>
</tr>
<tr>
<td>Collimating lens</td>
<td>Plano-convex lens, 50.8-mm diameter, 75-mm focal length, UV-grade fused silica</td>
<td>CVI Laser Corp.</td>
<td>(PLCX-50.8-38.6-UV)</td>
</tr>
<tr>
<td>Focusing lens</td>
<td>Plano-convex lens, BK7 glass, 100-mm diameter, 160-mm focal length</td>
<td>Comar Optics</td>
<td>(160-PQ-100)</td>
</tr>
<tr>
<td>Focusing lens</td>
<td>Plano-convex lens, 50.8-mm diameter, 75-mm focal length, UV-grade fused silica</td>
<td>CVI Laser Corp.</td>
<td>(PLCX-50.8-38.6-UV)</td>
</tr>
<tr>
<td>Fiber optic bundle</td>
<td>UV-visible fiber optic bundle, 3 meters long, 190-1100 nm spectral range, 200 µm fibers</td>
<td>Acton Research Corp.</td>
<td>(LG-455-020-3)</td>
</tr>
</tbody>
</table>
The emission that was observed in these experiments was expected to be either laser-induced plasma emission or “natural” fluorescence from the porcine corneal tissue. The term “natural” is used to describe fluorescence that is emitted as a natural result of absorption of the 193-nm excimer laser light into corneal tissue. Natural fluorescence is differentiated from laser-induced fluorescence, which in the context of this work refers to measurements taken when a second laser is used in conjunction with the ArF excimer laser to further excite the species of interest either in the tissue or in the ablation plume.

For all of the emission measurements, the ArF excimer laser was operated at a repetition rate of 4 Hz to accommodate the computer-controlled data acquisition system. The laser light was focused with a long focal length lens and the porcine eyes were positioned at the focal point to mimic clinical ablation. Operational parameters were set such that each spectrum recorded was the ensemble average of 25 consecutive laser pulses, light entering the spectrometer was dispersed onto the intensified CCD using a grating with 2400 grooves/mm, and 100 of 256 available rows of the CCD were binned for each spectrum. The signal acquisition of the CCD was appropriately timed using the iCCD pulse generator (see Table 2-1) to begin immediately before the excimer laser pulse, and the signal was integrated for 300 ns to collect emission during and after the laser pulse.

For this study, the excimer laser was used to ablate porcine corneal tissue, and emission from the surface of the eye or from any ejected materials that might be present during the signal integration time was recorded. The experimental matrix included emission measurements at two different values of laser energy in twelve different spectral windows. Ten of the spectral windows were continuous, starting with a window centered
at 225 nm and incrementing by 25 nm up to the final window centered at 450 nm. Measurements were also taken in spectral windows centered at 590 nm and 656 nm. For the purposes of this dissertation, specific spectral windows will be identified by their central wavelength values, a common practice in emission and fluorescence spectroscopy. In each window, replicate measurements were taken at nominal laser energy values of 3.5 and 5 mJ/pulse, which correspond to fluences of approximately 350 and 500 mJ/cm², respectively.

All replicate spectra recorded in a particular window and for a particular value of laser energy were averaged together. Ultimately, then, there were two spectra for each window corresponding to the two different laser energy values. The spectra for the ten continuous windows were pieced together for each pulse energy using the procedure outlined in the next section (Section 2.6.2) to yield a continuous spectrum spanning from approximately 200 to 460 nm.

It should be noted that two different sets of optics were used for these experiments, as shown in Table 2-7. The four-inch lenses (100-mm diameter, Comar Optics) were used for all measurements recorded in the 325-nm window and above, while the two-inch lenses (50.8-mm diameter, CVI Laser Corp.) were used for all measurements recorded in the 300-nm window and below. The four-inch lenses are actually preferable because they allow the collection of a great deal more light (i.e., fourfold increase in solid angle), which increases the measured signal. However, these lenses are made of BK7 glass, which transmits very little below 300 nm. The UV transmission of BK7 is so low that the geometric collection efficiency of the larger optics was completely neutralized and the signal was immeasurably small. Despite the lower geometric collection efficiency, the
highly transmitting nature of the two-inch lenses (UV-grade fused silica) led to a measurable and useful signal for the 300-nm window and below. However, a 225-nm long-pass filter was necessary to ensure that the laser light itself did not affect the signal via stray light, given the high transmission of the lenses at a wavelength of 193 nm.

2.6.2 Spectra Correction

Data collected in different spectral windows can only be compared in an absolute sense if they are corrected for dispersion effects. For a given spectral window, the central portion of the measured signal is typically characterized by a greater instrument throughput than the peripheral portions due to the dispersion efficiency of the spectrometer grating. Therefore, it is necessary to calibrate the collection system using a broadband light source with a known spectral irradiance distribution. Two different sources, which are listed in Table 2-8, were used for the current study. The Ocean Optics source was used to correct the spectra obtained in the 325-nm window and above, while the Optronic Laboratories tungsten lamp was used to correct the spectra obtained in the 300-nm window and below.

Table 2-8. Calibrated broadband light sources.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Manufacturer (Model or Part #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halogen lamp</td>
<td>Tungsten halogen white-light source, Optimized for VIS-NIR (360 nm-2 µm)</td>
<td>Ocean Optics (LS-1)</td>
</tr>
<tr>
<td>Tungsten lamp</td>
<td>1000-W FEL type lamp, Tungsten coiled-coil filament in quartz envelope,</td>
<td>Optronic Laboratories (F-215)</td>
</tr>
<tr>
<td></td>
<td>Calibrated for 250-2500 nm</td>
<td></td>
</tr>
</tbody>
</table>

In brief, spectra were recorded in each window with the relevant lamp as the light emission source and compared to the known irradiance distribution provided by the manufacturer. For the Optronic Laboratories source, a Planck distribution was fit to the manufacturer-provided calibration data to obtain values from 200 to 250 nm since they
were not provided. The comparisons between the recorded spectra and the known distributions provided the correction factors that were used to scale each of the porcine eye emission spectra, including those recorded in the 590-nm and 656-nm windows.

It was then possible to piece together the spectra from the ten continuous windows starting at 225 nm and ending at 450 nm. First, the spectra for the 225-nm, 250-nm, 275-nm, and 300-nm windows were combined into one spectrum and the spectra for the 325-nm, 350-nm, 375-nm, 400-nm, 425-nm, and 450-nm windows were combined into another spectrum. This separation was a result of the use of the two different calibration sources for these two sets of spectra. Figure 2-18 shows the corrected spectra for the 225-nm and 250-nm windows corresponding to ablation at 5 mJ/pulse.

![Figure 2-18. Spectra from the 225-nm and 250-nm windows, with the overlap region labeled. The two spectra have been corrected using a calibrated light source and are therefore on the same scale. The intensity scale remains arbitrary because the values do not represent absolute irradiances or intensities.](image-url)
Starting with the 225-nm and 250-nm spectral windows, the spectra were combined by averaging the data in the relevant overlap region. The spectrum from the 275-nm window was then added in the same manner and so on. The same process was done for the other set of spectra, beginning with the 325-nm spectral window and proceeding to the 450-nm window. Figure 2-19 shows the result of combining the two spectra from Figure 2-18.

![Figure 2-19. Spectrum that results from the combination of the spectra from the 225-nm and 250-nm windows. Data from the overlap region, shown in Figure 2-18, have been averaged to combine the spectra. Intensity remains arbitrary.](image)

The final task was to piece together the two already combined spectra, one spanning from the 225-nm to the 300-nm window and the other spanning from the 325-nm to the 450-nm window. It was necessary to scale one spectrum with respect to the other to account for the difference in the collection optics (two-inch and four-inch lenses). To accomplish this, data in the 656-nm window recorded for both sets of collection optics were compared to determine a flat scale factor between the two. Once this was
accomplished, all that remained was to combine the spectra by averaging the data in the overlap region. Ultimately, the entire procedure provided two spectra, corresponding to the two values of laser energy, that were continuous from approximately 200 nm to 460 nm, as well as corrected spectra in the 590-nm and 656-nm windows with the same relative scale.
CHAPTER 3  
CORNEAL HYDRATION STUDIES USING CONFOCAL RAMAN SPECTROSCOPY

An important factor that remains not well understood is the intraoperative role of corneal hydration in the laser’s ability to remove corneal tissue. Variations in the state of corneal hydration may help to explain unexpected outcomes after correctly performed refractive surgery, since the corneal ablation rate has been linked to corneal hydration. Average corneal ablation rates are 0.2 to 0.8 µm per laser shot for 193-nm ArF excimer lasers under clinically relevant conditions. However, current clinical excimer laser systems utilize an average ablation rate based on large patient populations, which may introduce significant error in refractive procedure outcomes. The methods described in Section 2.2 were used to measure corneal hydration as a function of time, the results of which are presented in this chapter.

3.1 Assessment of Raman Signal Linearity Using Water/Acetone Mixtures

Acetone (CH₃COCH₃) contains two CH₃ groups but lacks an O-H bond, while water contains only two O-H bonds. Accordingly, pure acetone will display an intense Raman peak near 2900 cm⁻¹ corresponding to the C-H stretching mode, and pure water will display a broad, prominent Raman band at 3400 cm⁻¹ (3100-3700 cm⁻¹) corresponding to the O-H stretching mode. These two bands are analogous to the collagen protein bands and water bands observed in the Raman spectra of corneal tissue, respectively. In pure solutions of either acetone or water, these two bands are mutually exclusive, while binary mixtures of acetone and water will exhibit both the C-H and the
O-H bands, with the relative intensities corresponding to their respective molar fractions. To confirm the quantitative use of the O-H to C-H Raman band intensities as a relative measure of hydration, experiments were performed in a series of water/acetone solutions ranging from pure water to pure acetone. A representative Raman spectrum corresponding to a mixture of 80% water and 20% acetone is shown in Figure 3-1.

![Raman spectrum](image)

**Figure 3-1.** Representative Raman spectrum for a mixture of 80% water (by volume) and 20% acetone, showing the C-H vibrational stretch of acetone and the O-H stretch of water.

The C-H band at 2922 cm\(^{-1}\) due to the acetone fraction and the O-H band at ~3400 cm\(^{-1}\) due to the water fraction are both well defined in the spectrum. The C-H and O-H Raman bands were analyzed to provide a quantitative measure of solution hydration. A baseline was fit for each spectrum and the absolute peak areas were calculated for each Raman band. The O-H and C-H Raman peak intensities are plotted as a function of acetone content in Figure 3-2.
As the acetone volume fraction increased from zero to 100%, the C-H peak area increased linearly and the O-H peak area decreased linearly. The data are characterized by a high degree of precision, with the average relative standard deviation equal to 0.4% and 0.8% for the O-H and C-H peaks, respectively. The data were fit with linear least-square lines (as shown in the plot) with resulting regression coefficients \( r \) greater than 0.999. It is noted that the two curve fits have nearly identical but inverted slopes, namely 1260 for the C-H peak data and negative 1257 for the O-H peak data.

### 3.2 Bovine Corneal Hydration Measurements

A series of experiments were performed to assess the transient state of corneal hydration corresponding to different surface treatments, as described in Section 2.2. Recall that the three surface treatments are designated control, MD (manual...
debridement), and LK (lamellar keratectomy). The eyes in the MD group were de-
epithelialized, while a flap was cut to expose the corneal stroma of each eye in the LK
group. For the current study, relative hydration is defined as the ratio of the integrated O-
H Raman band to the integrated C-H Raman band. It is noted that earlier studies support
the use of O-H to C-H Raman bands as a quantitative measure of hydration (Bauer et al.,
1998, 1999). Quantitative data are presented directly as the O-H to C-H ratios; however,
for brevity, additional discussion may refer simply to corneal hydration, which is
considered analogous to the reported O-H to C-H ratios.

Figure 3-3 shows two representative Raman spectra, one recorded from a de-
epithelialized cornea at the outset of the forced nitrogen flow experiment, and one
recorded from the same cornea after five minutes of exposure to nitrogen flow.

Figure 3-3. Baseline-corrected Raman spectra from a de-epithelialized bovine cornea
 corresponding to the outset (upper spectrum) of forced flow drying, and
 following five minutes of exposure to forced flow drying (lower spectrum).
As demonstrated in the spectra, the peak area of the C-H band (2900 cm\(^{-1}\)) remained relatively constant while the O-H band (3100-3700 cm\(^{-1}\)) intensity decreased markedly after five minutes. The O-H to C-H Raman peak area ratio decreased from 8.7 to 5.0, corresponding to a 43% decrease in relative hydration after five minutes of drying.

The Raman-based hydration data for all bovine cornea experiments are summarized in Figures 3-4 and 3-5, which correspond to the quiescent air drying and forced flow drying conditions, respectively. For all data analysis, the O-H/C-H peak ratios were normalized with respect to their initial values for each cornea. For each experimental group, the normalized values were then averaged corresponding to each drying time, thus each data point represents an average of five corneas (\(N=5\)) from five different bovines.

![Figure 3-4. Normalized ratio of integrated O-H band intensity to integrated C-H band intensity of bovine corneal tissue as a function of time for exposure to quiescent air drying for the three treatment groups (control, MD, and LK). The error bars represent ± one standard deviation (\(N=5\)).](image-url)
Figure 3-5. Normalized O-H/C-H ratio of bovine corneal tissue as a function of time for exposure to forced flow drying for the three treatment groups (control, MD, and LK). The O-H/C-H ratio is normalized by the initial, pre-flow (time equals negative 0.5 min) value of the ratio. The error bars represent ± one standard deviation (N=5).

For all experimental conditions, the average relative standard error was 6.3%. For the forced flow data, the normalizing initial O-H/C-H ratio was recorded thirty seconds prior to the nitrogen flow initiation. Therefore, in Figure 3-5 the O-H/C-H ratios corresponding to zero flow time correspond to the actual first ten seconds of nitrogen flow, and the starting ratio corresponds to a time of negative thirty seconds.

Several key features characterize the corneal hydration data shown in Figures 3-4 and 3-5. In the case of quiescent air drying, the O-H/C-H peak ratio of both the control corneas and the MD corneas remained relatively constant, decreasing to 93.4% (5.6% relative standard deviation, rsd) and 94.1% (3.8% rsd) of the initial values, respectively. In contrast, the LK corneas revealed a more significant decrease in hydration over six
minutes, ending with an average O-H/C-H peak ratio equal to 81.1% (3.5% rsd) of the initial hydration values. For the case of forced nitrogen gas drying, all three corneal treatments revealed a marked decrease in the O-H/C-H peak ratio over time, with the MD and LK eyes experiencing much more pronounced decreases than the control eyes. The statistical significance of the data is discussed in Section 3.3. In concert, the Raman data demonstrate a marked difference in the extent of corneal dehydration realized with both the different drying scenarios and the different surface treatments.

With all data shown in Figures 3-4 and 3-5 normalized to an initial value of unity, the final hydration level (O-H/C-H ratio) indicates the degree of reduction in corneal tissue hydration over the course of the six-minute drying period. For comparison of all experimental conditions, the normalized O-H/C-H ratios are summarized in Figure 3-6 after three minutes and six minutes of drying.

![Normalized O-H/C-H ratio](image)

**Figure 3-6.** Normalized O-H/C-H ratio of the bovine corneas after three minutes and six minutes of exposure to quiescent air or forced flow drying for the three treatment groups (control, MD, LK). The error bars represent one full standard deviation (N=5).
The average final decreases in corneal hydration for the different treatment methodologies, as measured by the percent decrease in O-H/C-H ratio after six minutes, are summarized in Table 3-1.

Table 3-1. Final percent decrease in corneal hydration (± standard deviation, N=5) with respect to the initial hydration state.

<table>
<thead>
<tr>
<th>Cornea Treatment</th>
<th>Quiescent Air</th>
<th>Forced Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.6% ± 0.4%</td>
<td>25.5% ± 1.7%</td>
</tr>
<tr>
<td>MD</td>
<td>5.9% ± 0.2%</td>
<td>40.7% ± 1.7%</td>
</tr>
<tr>
<td>LK</td>
<td>18.8% ± 0.7%</td>
<td>40.7% ± 8.5%</td>
</tr>
</tbody>
</table>

3.3 Statistical Comparisons of Hydration Measurement Results

Using a two-sided Student’s t-test with a 99% confidence level (α=0.01), there is no statistically significant difference between the control and the MD groups exposed to six minutes of quiescent air drying (p=0.816). In contrast, the LK group revealed a statistically significant reduction in the O-H/C-H ratio in comparison to the control corneas (p=0.00486) and the MD corneas (p=0.000219) after drying for six minutes in quiescent air.

Using the same statistical test, it is concluded that there is no difference between the relatively large decrease in the O-H/C-H ratio realized with the MD and LK treatment groups (p=0.999) after six minutes of forced nitrogen flow. There is, however, a statistically significant difference in the decrease in the O-H/C-H ratio between the control corneas and the MD corneas (p=0.0051) under forced drying conditions. Under the same forced flow conditions, a similar difference is reported between the control corneas and the LK corneas (p=0.0538) using a confidence level of 94% (α=0.06). The relaxed confidence level reflects the somewhat larger standard deviations realized with the LK treatment protocol.
In comparing the effects of quiescent and forced flow drying for a given corneal treatment, the final O-H/C-H ratios are statistically different for the control eyes (p=0.00109) and the MD eyes (p=4.16E-7) with a confidence level of 99% (α=0.01). In comparing quiescent and forced flow for the LK treatment, the two drying scenarios are also statistically different (p=0.0181) for a confidence level of 98% (α=0.02).

While the above analysis details the degree to which the O-H/C-H Raman ratio decreases after six minutes for the various experimental conditions, it is also useful to assess the change observed in a shorter time interval. In the present study, rather significant decreases in corneal hydration, as measured by the O-H/C-H ratio, were evident in as little as one minute, as observed in Figures 3-4 and 3-5, for the different experimental conditions. After 1.5 minutes of forced gas flow, the average O-H/C-H ratio for the control, MD, and LK groups were reduced to 91.9%, 82.4%, and 78.4%, respectively, of their initial values. Using a two-sided Student’s t-test, the decrease in the O-H/C-H ratio was statistically significant for the MD corneas (p=0.00079) at a confidence level of 99% (α=0.01) and for the LK corneas (p=0.0761) at a confidence level of 92% (α=0.08) after only 1.5 minutes with respect to the control value. As a final item concerning Figures 3-4 and 3-5, it is worth noting that the rate of decrease in the O-H/C-H ratio for all six experimental conditions was nearly linear albeit with a few aberrant data points. There was, however, a slight upturn in the O-H/C-H ratio near the end of the drying sequence, perhaps indicating a “leveling off” of the dehydration effects beyond approximately five minutes.

While all of the hydration data presented above were normalized with respect to the initial O-H/C-H Raman peak ratios, it is useful to examine the absolute values of the
ratios associated with the various treatments. All of the bovine eyes (control, MD, and LK) were obtained in pairs from freshly sacrificed cows. For a given bovine pair, each eye was treated identically and subsequently subjected to either quiescent or forced flow drying. The initial Raman spectrum of each cornea was used as a reference to calculate the initial O-H/C-H ratio, which was then used for the normalization of all subsequent data. The absolute O-H/C-H ratio for each of the corneas at the initial, physiologically hydrated condition is presented in Figure 3-7.

![Figure 3-7](image-url)

Figure 3-7. The absolute initial value of the O-H/C-H ratio of bovine corneal tissue at the outset of each experiment for all groups (control, MD, and LK). Each pair of shaded bars represents paired eyes from a single bovine.

Each bovine sample number contains a pair of shaded and unshaded bars corresponding to a pair of eyes from a single cow. No attempt was made to identify the left and right eyes. As observed in the figure, the initial O-H/C-H peak ratio was similar for both eyes in a given pair, and for all eyes in a given treatment group, notably so with
the control and MD groups. The greatest variability in initial hydration existed for the
LK group. This is attributed to the variability from eye to eye in the depth of the corneal
flap, as well as to the somewhat rough surface texture of the resulting corneal stroma, due
to the manual cutting of corneal tissue. The resulting surface microstructures can be
characterized by high surface-to-volume ratios, which can lead to local dehydration in
very short time-scales in view of the earlier results. With this in mind, a relatively large
variation in absolute hydration, as assessed using the micro-Raman system, is expected
for the flap treatment group.

A monotonic increase in the O-H/C-H ratio was noted as the Raman measurements
progressed from the surface of the cornea to deeper into the cornea stroma after removal
of the flap. On average, the initial O-H/C-H ratio was 7.56 ± 0.46 for the control eyes,
9.03 ± 0.58 for the MD group, and 13.61 ± 3.13 for the LK group (N=10 for each group).
A two-sided Student’s t-test with a confidence level of 99% (α=0.01) revealed
statistically significant differences between the control eyes and MD eyes (p=8.80E-6),
between the MD eyes and the LK eyes (p=0.000874), and between the control eyes and
the LK eyes (p=0.000158).

In other words, the control eyes, which were left untouched and therefore
investigated on the anterior portion of corneal tissue with intact epithelium, had the
lowest level of relative hydration at the initial measurement. The MD eyes, which were
investigated on the anterior portion of de-epithelialized corneal tissue, were noticeably
more hydrated initially than the control eyes. Finally, the LK eyes, which were
investigated on the surface of the exposed corneal stroma, were noticeably more hydrated
initially than both the control eyes and the MD eyes. In conclusion, corneal hydration as
measured by the Raman technique increases with progressively deeper locations in corneal tissue. These results are in excellent agreement with the findings reported by Bauer et al. (1998), namely that the level of corneal hydration (O-H/C-H ratio) increased as the cornea was probed from anterior to posterior.

3.4 Discussion and Conclusions

The primary conclusions of the Raman hydration studies are summarized as follows:

1. The confocal Raman technique is an effective way to measure relative corneal hydration using the C-H protein bands and the O-H water bands.

2. For all treatment groups, the relative state of corneal hydration, as measured by the ratio of the integrated O-H peak to the integrated C-H peak, decreased with time. The decrease in corneal hydration is statistically significant within the first 90 seconds of corneal exposure to quiescent air or forced flow drying.

3. For all treatment groups, the O-H/C-H ratio decreased to a greater extent and at a greater rate when the cornea was exposed to a forced flow of nitrogen gas as compared to quiescent air drying.

4. The O-H/C-H ratio varied depending on the surface treatment method. Removing the epithelium (i.e., MD) did not decrease the O-H to C-H ratio relative to controls for quiescent air drying, but decreased the ratio relative to controls when corneas were exposed to forced flow drying. Creating a corneal flap (i.e., LK) decreased the ratio relative to controls for corneas exposed to quiescent air and forced flow drying.

5. The absolute degree of corneal hydration, as measured by the O-H/C-H ratio, increased as measurements were made deeper into the cornea.

In addition to the conclusions enumerated above, specific comments are offered with respect to the current findings, including discussion of the relative physical processes associated with transient changes in corneal hydration, and the clinical relevance of the present findings.
3.4.1 Linearity and Suitability of Raman Signal

The water/acetone Raman study demonstrated the quantitative nature of the O-H and C-H Raman peak area ratios as a metric for relative hydration. The Raman data for these experiments were characterized by a high degree of precision, and yielded an ideal inverse relationship between the two response functions (i.e., Raman band peak areas) for binary mixtures of the two compounds. The water/acetone data support the current use of the O-H to C-H Raman band ratio as a quantitative measure of relative corneal hydration.

This is consistent with earlier studies in which the O-H to C-H ratio was reported as a quantitative metric for water content in an albumin model and for changes in corneal hydration following application of a dehydrating drug (Bauer et al., 1998, 1999). As such, the confocal Raman system is well suited for the assessment of transient changes in corneal hydration corresponding to different surface treatment methodologies. For the remainder of the discussion, it should be understood that relative corneal hydration refers to the O-H/C-H Raman peak ratio.

3.4.2 Interpreting the Physical Processes and Boundary Conditions Related to Corneal Hydration

The bovine eye experimental data support the present conclusion that significant changes in corneal hydration are realized under different drying conditions and surface treatment methodologies. More significantly, exposure of the exposed corneal stroma (LK group) to a forced gas flow condition can induce a 10-20% decrease in corneal hydration in as little as two minutes.

With these findings in mind, additional comments are offered with regard to the role of induced airflow velocity in corneal dehydration. Using the slope of the O-H/C-H Raman ratio as a function of time (Figures 3-4 and 3-5), the rates of corneal dehydration
were evaluated in the temporal region between 1 and 3.5 minutes of drying time, a region that is characterized by an approximately linear decay. For the control and MD corneas, the rate of dehydration was increased by a factor of 7.4 and 10.6, respectively, with forced flow drying as compared to the quiescent air drying. In contrast, for the LK group, the rate of corneal dehydration only doubled with forced flow as compared to quiescent air drying. Note, however, that the LK baseline quiescent drying case was significantly greater than the quiescent drying realized with either the control or MD cases.

In concert, the dehydration rate data offer insight into the relative importance of water diffusion within the corneal tissue and mass transfer from the corneal tissue to the surrounding gas. The data suggest that the rate of dehydration is significantly influenced by the rate of water diffusion to the corneal surface under forced flow conditions. This finding would explain the more significant increase in the rate of dehydration (forced flow to quiescent) with the MD corneas as compared to the control corneas. The epithelial cells are expected to present an additional barrier to water diffusion to the cornea surface. As a result, the forced flow condition is limited for the control case to a dehydration rate that is less than that observed with the MD corneas.

When comparing the MD and LK conditions under forced drying, it is reasonable to conclude that the presence of the relatively thick bovine basement membrane in the MD corneas does not appear to offer an appreciable additional resistance to water diffusion to the surface as compared to the corneal stroma exposed by flap removal. Hence, these two treatment conditions were characterized by similar dehydration rates under forced flow drying, presumably due to the overall limitation of water diffusion
through the corneal tissue to the surface. These findings suggest a “saturation” effect with forced flow dehydration. Hence additional increases in air velocity contribute little to the rate of corneal tissue dehydration, which is controlled primarily by the rate of water diffusion through the corneal stroma.

In contrast to forced flow drying, quiescent drying is more likely limited by mass transfer from the corneal surface to the bulk surrounding air. This conclusion is consistent with the similar dehydration rates observed with quiescent air drying for the control and MD corneas. The greater rate of corneal dehydration observed with the LK group under quiescent drying is assumed to be due to an increase in available corneal stromal surface area, and to an increase in the absolute surface water content secondary to the slight hydration gradient realized within the corneal tissue (see Figure 3-7).

The presence of a pronounced hydration gradient, with hydration increasing with depth from the anterior surface, directly couples to the transport of water to the corneal surface. As noted above, the LK data are characterized by a larger experimental variability as compared to the control and MD data. It is expected that a thicker flap will produce a corneal surface characterized by increased hydration at the interface, due to the hydration gradient, thereby promoting water diffusion within the cornea and an enhanced rate of dehydration. In view of these comments, the range of flap thickness (100 to 250 μm) in the current study is most likely a significant contributor to the variability in the LK results.

Final comments are offered with respect to the effects of boundary conditions on corneal dehydration. Relative humidity of the surrounding environment may affect the rate of corneal dehydration. Accounting for the entrainment of ambient air via diffusion
into the forced jet flow, the relative humidity of the forced flow at the cornea interface is estimated to be 75% of the ambient air value, which was consistently at about 50%. If the relative humidity of the nitrogen gas flow were increased to 50%, the present dehydration rates would be expected to decrease by about 20% for gas-transfer limited dehydration, notwithstanding the above discussion regarding the overall limitation of water diffusion through the corneal tissue to the surface, which is unaffected by ambient humidity.

3.4.3 Clinical Relevance

These Raman studies suggest that changes in the corneal hydration state may occur with common excimer laser refractive surgery preoperative treatment methodologies, namely epithelium removal and lamellar keratectomy. Given the generally accepted dependence of excimer laser corneal ablation rates on the absolute state of corneal hydration, such dynamic changes in corneal hydration may subsequently affect the outcome of clinical refractive procedures.

The exact mass transfer problem of corneal drying is a complex problem involving mass diffusion in both the corneal tissue and gas stream, coupled with the fluid dynamic boundary layer flow. A solution for mass transfer based on a constant surface water content, binary mass diffusion of water vapor in air, and a simplified set of boundary flow equations yields a mass transfer rate proportional to the square-root of the free stream gas velocity (Skelland, 1974). Based on this analysis, the nominally eightfold decrease in the velocity of the forced airflow realized with a clinical aspirator (as compared to the current study) is expected to result in an approximately threefold decrease in corneal dehydration rate with respect to the current data reported for the forced nitrogen jet. However, as discussed below, the current data suggest a saturation
effect for forced flow dehydration; hence surface mass transfer (i.e., from corneal tissue to the gas stream) may not completely control the cornea flap dehydration conditions under all forced flow conditions. Clearly additional research is necessary to further elucidate the important mechanisms of corneal dehydration under more clinically relevant conditions. Nonetheless, the current data emphasize the importance for surgeons to standardize the time between lifting the LASIK flap and initiating excimer laser ablation, particularly if the vacuum aspiration tube has been put in place. The importance of standardization was recognized in the protocol used for an early study on LASIK (Pallikaris et al., 1999).

It is also noted that clinical studies have reported variability with respect to the mean flap thickness ranging from ± 20 to ± 50 µm, which may contribute to variations in clinical outcome (Buratto, Ferrari & Rama, 1992; Spadea, Cerrone, Necozione & Balestrazzi, 2002). Furthermore, human corneas are about 30% thinner than bovine corneas, hence human corneas may have a greater corneal hydration gradient, leading to enhanced dehydration rates and greater variation in dehydration rates with changing flap thickness. Relative humidity of the surrounding environment may also affect the rate of corneal dehydration, as noted above. Humidity levels reported in the literature regarding LASIK studies range from about 40 to 50%, and while various clinical systems may recommend humidity levels, overall the important point is that the humidity should be as constant as possible from procedure to procedure (Doane et al., 1996; Krueger et al., 1993; Walter & Stevenson, 2004).

Finally, surface hydration of the cornea may be directly affected by excimer laser ablation, which may significantly affect the boundary conditions. Oshika et al. (1998)
suggested that shock waves generated during laser ablation might cause moisture to pool centrally within the ablation zone. They further concluded that this pooling contributed to the formation of steep central islands, a condition where significantly less tissue is ablated locally. Maldonado-Codina, Morgan and Efron (2001) reported temperature increases of nearly 9°C over the course of various PRK procedures ranging in correction from two to ten diopters. Temperature changes resulting from laser ablation will certainly modify the boundary conditions for the mass transfer problem, which may affect any dynamic changes in corneal hydration.

In conclusion, this study demonstrates the potential utility of using Raman spectroscopy to analyze the cornea in vivo with the goal of better understanding excimer laser treatment methodologies and it elucidates changes in corneal hydration that occur with time. Specifically, the study shows that corneal hydration varies depending on the exposure time, type of corneal exposure (quiescent air or forced flow drying), and type of surface treatment (MD or LK). The data offer possible insight into unexpected refractive surgical outcomes for successfully administered laser treatments. This study further reinforces the importance of consistency in surgical technique, particularly with respect to the initiation of excimer laser treatment after lifting the LASIK flap, or removing the epithelium for PRK, and the positioning of the vacuum tube. Ultimately, it is expected that a better knowledge and understanding of the underlying physics and corneal physiology will lead to enhanced precision and accuracy of laser refractive surgery.
Despite the relatively high degree of accuracy and precision of clinical LASIK and PRK systems in reshaping the cornea, one important limitation remains. While the ablation rate may differ from patient to patient, between eyes for a given patient, and perhaps even over the course of treatment for a given eye, no means currently exist for real-time correction to refractive treatment algorithms to account for these variations. Efforts to further enhance the precision of laser refractive procedures can benefit from a better understanding of the corneal ablation process, which in turn requires a means to precisely measure ablation rates. The methods presented in Section 2.3 were used to quantify the ablation rate of corneal tissue, and the results are presented in this chapter. Additional data are presented for PMMA ablation to support the current approach.

### 4.1 Validation of Method Using PMMA

Recall from Section 2.3 that a method to measure the depths of ablation craters using white-light interferometry has been developed. For various reasons that will be discussed in detail later in this chapter, however, bovine corneal tissue cannot be measured directly using a white-light interferometry. This obstacle led to the use of crater molds or impressions that can be measured using a white-light interferometer. Polymethyl methacrylate, or PMMA, was used to validate the use of wax molds because the actual ablation craters themselves can be measured directly with the interferometer and compared to the results obtained from the wax molds.
4.1.1 Results from PMMA Measurements

The direct comparison of the resulting cross-sections from actual craters and from corresponding wax impressions enabled an assessment of the fidelity of the wax impressions in capturing the profiles of the actual ablation craters. Figure 4-1 presents the depth profile plot of a PMMA crater resulting from 28 laser pulses and the inverted depth profile plot of the wax impression corresponding to the same crater. The profiles shown in Figure 4-1 are typical of all of the results, supporting the conclusion that the depth profile plots of the wax impressions provide an excellent representation of the actual PMMA crater profiles.

![Figure 4-1](image.png)

Figure 4-1. Representative cross-sectional profiles of a PMMA ablation crater resulting from 28 laser pulses and the corresponding inverted wax impression. Both profiles have the same scale and have been linearly shifted.

From the depth profiles of the PMMA craters and wax impressions, ablation depths were determined using two different methods as described in Section 2.3. For a given number of laser pulses, three replicate ablation craters were created ($N=3$), and the
average ablation depth was calculated for both the crater and the corresponding wax impression. Ablation rates were then calculated based on each of these individual crater depth measurements, and Method 1 and Method 2 (see Section 2.3.1) produced ablation rates that differed by an average of less than 1%. Therefore, all reported results were determined using only Method 2 to determine the ablation depths.

The results of the ablation depth measurements are plotted in Figure 4-2 based on the ablation craters and corresponding wax impressions, with the error bars representing plus or minus one full standard deviation. There is a clear monotonic linear increase in ablation depth as the number of laser pulses increases. The corresponding linear regression lines for the PMMA ablation craters ($r=1$) and for the wax impressions ($r=0.9995$), respectively, are $y=-0.017+0.471x$ and $y=-0.043+0.481x$.

![Figure 4-2](image)

Figure 4-2. Ablation depth as a function of the number of ablating laser pulses for both PMMA ablation craters and corresponding wax impressions. Each data point is the mean depth ($N=3$) and the error bars represent ± one standard deviation. Regression lines are included.
The slopes of the regression lines are a measure of the ablation rate of PMMA. For the PMMA ablation crater data, an ablation rate of 0.47 µm/pulse is reported. For the corresponding wax impression data, an ablation rate of 0.48 µm/pulse is reported. An ablation rate was also calculated based on each individual depth measurement for both the PMMA craters and the wax impressions. A total of twelve measurements (three craters for each of four total number of laser pulses) of ablation rate were then averaged together for the PMMA craters and for the corresponding wax impressions. The resulting ablation rates were 0.47 ± 0.01 µm/pulse and 0.48 ± 0.01 µm/pulse for the PMMA craters and wax impressions, respectively, which are in exact agreement with those obtained from the slopes of the regression lines as reported above.

The ablation rates were also determined as a function of the number of laser pulses, hence based on the average of the three craters only. The results of these calculations are shown in Table 4-1 with appropriate standard deviations, as well as the percent difference between the rates as determined by PMMA craters and by wax impressions. It is noteworthy that the individual ablation rates are consistent regardless of the depth of ablation.

<table>
<thead>
<tr>
<th># Laser Pulses</th>
<th>Ablation Rate (µm/pulse) based on PMMA Craters</th>
<th>Ablation Rate (µm/pulse) based on Wax Impressions</th>
<th>Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>0.47 ± 0.007</td>
<td>0.47 ± 0.008</td>
<td>1.02 ± 2.242</td>
</tr>
<tr>
<td>21</td>
<td>0.47 ± 0.010</td>
<td>0.48 ± 0.015</td>
<td>1.89 ± 3.724</td>
</tr>
<tr>
<td>28</td>
<td>0.47 ± 0.009</td>
<td>0.48 ± 0.015</td>
<td>2.13 ± 3.704</td>
</tr>
<tr>
<td>35</td>
<td>0.47 ± 0.011</td>
<td>0.48 ± 0.018</td>
<td>1.88 ± 4.388</td>
</tr>
</tbody>
</table>

4.1.2 Statistical Analysis of PMMA Measurements

A two-sided, paired Student’s t-test was performed to compare the ablation depths based on direct measurement of PMMA craters and those based on the wax impressions.
These measurements, which are plotted in Figure 4-2, were analyzed with a paired test because of the progressive nature of the ablation depths. It was found that the differences between the two sets of depth measurements were statistically insignificant (p=0.055) at 95% confidence (α=0.05). This provided quantitative support for the qualitative observation that the wax impressions accurately mimicked the PMMA craters, which was qualitatively illustrated in Figure 4-1.

Recall that the ablation depth was calculated using a depth profile that was the average of three individually defined profiles. To assess the repeatability of this procedure, a second iteration of selecting the cross-sectional profiles was performed for each ablation crater and wax impression and the percent difference was calculated, using the first iteration as the reference value, for each depth measurement. For the PMMA craters and corresponding wax impressions, the ablation depths measured from the second iteration differed from those of the first iteration by an average of 1.1% and 1.7% (absolute values), respectively. The differences between iterations were found to be statistically insignificant both for the PMMA ablation craters (p=0.44) and for the wax impressions (p=0.39) at a confidence level of 95% (α=0.05). This result supports the current procedure of defining the cross-sectional profiles in triplicate and calculating the ablation depth from the average profile.

Lastly, a paired, two-sided Student’s t-test with a confidence level of 95% (α=0.05) was performed to compare the individual ablation rate measurements based on the measured depths for the PMMA craters and wax impressions. The difference between them was found to be statistically insignificant (p=0.056), providing a direct validation of the wax impression method for the determination of ablation rates.
4.1.3 Depth Profile Issues

The discussion presented here is related to the profiles shown in Figure 4-1, but is meant to highlight features or issues that were observed consistently throughout the PMMA data. In Figure 4-1, the profile of the PMMA crater has a smooth and continuous contour with a relatively flat bottom and smooth transitions to the untouched surrounding material at the right and left edges. In contrast, the inverted profile of the wax impression, while maintaining a similar overall trend to the PMMA, displays localized spikes, especially near the “shelves” or outside edges. These spikes were considered a real result of features on the wax surface and are most likely due to ablation debris adhering to the wax surface. Essentially, the wax “cleans” the ablation crater, resulting in relatively smooth PMMA crater profiles.

These debris features are evidence of the relatively high spatial and depth resolution of the interferometer technique. The spikes made it difficult to use Method 1 to determine ablation depths, because it was necessary to individually mask these features on each of the profiles. It was considered useful that there was basically no difference between the results obtained using Methods 1 and 2, since it was then possible to quickly and yet reliably use Method 2 to determine ablation depths and rates. The spike features had little effect on determination of the profile depth as determined by Method 2, as these features were ignored and the overall profile contour was used as a guide.

It may also be apparent from Figure 4-1 that the left and right shelves of the craters are not at the same horizontal level. This is a real result that has two physical explanations. First, no surface is completely flat, no matter how smooth and flat it may appear. The interferometer presents results at the sub-micron scale, and it is unrealistic to expect that the materials used in this study should appear flat when viewed at the
microscopic level. Second, the measured samples were not purposely leveled when
placed under the interferometer, so there is no absolute horizontal reference plane.
Therefore, it should not be surprising that the areas surrounding the ablation craters are
not in the same horizontal plane.

There was initial concern about the consistency and precision in selecting cross-
sectional profiles that best bisect the ablation crater because of the nature of the
topographical maps of the craters. In the software provided with the interferometer, the
depth is represented through a continuous scale of color shades, making it difficult to
visually determine the exact point at which a crater is deepest. However, when this task
was performed in triplicate, it was difficult to distinguish the profile plots from each
other, and it was concluded that averaging three cross-sectional profiles was a reliable
and consistent procedure. The repeatability of choosing three cross-section profiles,
averaging them together, and determining the crater depth using Method 2 was further
assessed by repeating the procedure a second time. The differences between the depth
values obtained from the first and second iterations were random in nature, and the
average absolute percent difference was less than 2% for both the PMMA ablation craters
and the wax impressions.

4.1.4 Interpretation of Ablation Depth Regression Lines

The PMMA ablation depth data, as presented in Figure 4-2, ranged from about 6.5
µm for 14 laser pulses to about 16.5 µm for 35 laser pulses. Linear least-squares fits
(with near-unity correlation coefficients) are extrapolated to the origin in Figure 4-2. It is
noted that as the number of laser pulses increases, the trend lines are observed to diverge
slightly. This trend of divergence is presumably due to the accumulation of ablation
debris around the crater edge, which subsequently adheres to the wax providing a somewhat deeper crater. However, the divergence is not considered significant from a practical standpoint for the current number of laser pulses investigated.

The two relevant parameters of the regression lines in Figure 4-2 are the y-intercepts and the slopes. First, the y-axis intercepts provide information about any ablation incubation effect. The y-axis intercepts for the regression lines are negative 0.017 (PMMA) and negative 0.043 (wax), respectively. The data support the absence of an appreciable “incubation” effect for PMMA at the current average fluence of 350 mJ/cm$^2$. Researchers are in general agreement that PMMA incubation is only significant at low fluence. Previous work investigating the ablation rates of polymers found that for laser fluences at or near the ablation threshold, ablation of PMMA is initiated only after an accumulation of 5-20 laser pulses (Ediger et al., 1993b; Pettit, Ediger & Weiblinger, 1991; Srinivasan et al., 1990). The reported incubation ranges from 18 pulses at a fluence of 115 mJ/cm$^2$ to only 2 pulses required for ablation onset at 265 mJ/cm$^2$ (Pettit et al., 1991).

The exact cause and nature of this incubation effect has not been fully determined. Regardless, researchers are in general agreement that incubation is only significant at low fluences for PMMA. In the current study, the average fluence of 350 mJ/cm$^2$ was considered high enough to eliminate any incubation effects. This assumption was clearly supported by the results presented in Figure 4-2, namely the intersection of the regression lines with the origin. The lack of an incubation effect with the ablation rate data is corroborated by the observation that a pronounced audible snap was heard for every laser pulse, including the first.
While the y-axis intercepts provide information about incubation effects, the slopes of the regression lines give a measure of the ablation rate, in terms of depth ablated per laser pulse. As reported in Section 4.1.1, the slopes of the regression lines, reported to two significant figures, agree exactly with the ablation rates calculated directly from average depth measurements.

A final observation is made regarding the absolute accuracy of the ablation rate of PMMA, namely that the measured values agree very well with previously reported values. Srinivasan et al. (1986) measured PMMA ablation depths using a Tencor Alpha step profilometer and reported ablation rates near 0.45 \( \mu \text{m/pulse} \) at fluences of approximately 300-400 mJ/cm\(^2\). Using the microscope focusing technique described in Section 1.6, Costela et al. (1995) reported that the ablation rate of PMMA increases with increasing fluence until it saturates at a value of 0.5 \( \mu \text{m/pulse} \) at about 500 mJ/cm\(^2\). These values are in excellent agreement with the current ablation rate of 0.47-0.48 \( \mu \text{m/pulse} \) for an average fluence of about 350 mJ/cm\(^2\).

4.2 Measurement of Corneal Tissue Ablation Rate

4.2.1 Results

The ablation craters in the bovine cornea, as measured with the wax impressions, revealed the same essentially rectangular cross-sectional shape observed with the PMMA craters. As with the PMMA experiments, two wax impressions were created for each ablation site, which are referred to as the first and second wax impressions. A typical cross-sectional depth profile is presented in Figure 4-3 for the wax impression (after inversion) of a corneal tissue crater corresponding to 24 laser pulses.
Figure 4-3. Cross-sectional profile of an inverted wax impression corresponding to a bovine corneal ablation crater. The crater was the result of ablating the tissue with 24 laser pulses.

The profile is similar in shape to those recorded for the PMMA, although the craters are deeper for a similar number of shots and the crater floor is clearly not as flat or well defined. Note also that the edges of the profile are somewhat smoother than the wax impressions obtained from PMMA. Despite these differences, the same method was used for determining ablation depths for the bovine eyes as for the PMMA, according to the previously described protocol of Method 2. The measured ablation depths are plotted in Figure 4-4 as a function of the number of laser pulses delivered, with each point representing the average value for up to seven bovine eyes and the error bars representing plus or minus one full standard deviation. The reported ablation depths for the 16, 24, 32, and 40-shot craters are average values based on 4, 5, 7, and 5 individual bovine eye measurements, respectively.
Figure 4-4. Ablation depth as a function of the number of ablating laser pulses for wax impressions corresponding to bovine corneal ablation craters. Each data point is the mean depth and the error bars represent ± one standard deviation.

As shown in Figure 4-4, a regression line (r=0.996) was fit to the bovine eye data, and its corresponding equation is \( y = 0.904x \). The slope of this regression line is an indication of the corneal tissue laser ablation rate; therefore, an ablation rate of 0.90 \( \mu \text{m}/\text{pulse} \) is reported for intact bovine corneal tissue at the current laser fluence. The depth measurements for each individual crater were also used to calculate ablation rates, which on average yielded a value of 0.87 ± 0.11 \( \mu \text{m}/\text{pulse} \). This is in excellent agreement with the value based on the slope of the regression line.

The 40-shot craters were also used to assess any differences obtained in determining the ablation depths when using either the first or second of the two wax impressions. The average depth of the 40-shot craters based on measurements of the first wax impressions was 38.3 ± 2.2 \( \mu \text{m} \). For the second wax impressions, the average depth
was 37.5 ± 2.3 µm. A two-sided Student’s t-test with a confidence level of 95% (α=0.05) was performed to compare these two measurements, and the approximately 2% difference between them was found to be statistically insignificant (p=0.58). This comparison was made for a total of five ablation (N=5) sites rather than seven due to data dropout, in which the three-dimensional topographical image was incomplete due to the failure to achieve a full interferometry measurement over the relatively steep impressions. This phenomenon is discussed in detail later in this chapter.

Confocal Raman hydration measurements confirmed that there was no appreciable change in corneal hydration, as measured by the ratio of the integrated OH-Raman band to the integrated CH-Raman band, from the time immediately prior to ablation of the first site to the time immediately following ablation of the final site of each eye.

4.2.2 White-Light Interferometry Issues With Respect to Corneal Tissue

There are several issues that make it difficult to directly investigate corneal tissue ablation craters using an interferometer, providing the motivation for the current development of the wax impression technique. First and foremost, the ablated eye must be preserved in its exact condition as at the time of ablation prior to measurement with the interferometer. During any introduced time interval, changes in hydration (either dehydration or hydration due to water diffusion to the corneal surface) can distort the cornea as a whole and therefore distort the shape and size of the ablation crater. Changes in corneal topography, even on the scale of microns, can significantly affect measured ablation rates for crater dimensions on the order of those in the present study. In addition, removal of the target eye from the ablation experiment may distort the curvature of the corneal surface and therefore the ablation crater. Transportation of the eye from the
location of the ablation experiments to the interferometer instrument may exacerbate these effects.

A second issue is the highly transparent nature of corneal tissue. The white-light interferometer directs light at the target surface and records the subsequent interference fringes in the reflected light. A complete measurement requires a large amount of reflected light, which is difficult to achieve from highly transparent materials such as corneal tissue.

For these reasons, white-light interferometry is not a viable approach for the direct measurement of corneal ablation craters. The method of forming a representative impression using heated paraffin wax was developed to overcome these limitations by exploiting the very low viscosity of liquid wax and the rapid time for the liquid wax to solidify upon contact with the corneal tissue. The PMMA study verified the ability of the wax impressions to accurately represent the size and shape of actual ablation craters. It also verified the reliability of using a visual, manual method of determining ablation depths (Method 2). For the eye study, it was determined that the best way to represent the crater was to apply the liquid wax immediately after the ablation without disturbing the eye in any way. This was readily achieved by directing the laser beam vertically downward onto the target eyes, which were positioned facing upward to create a horizontal surface for direct application of the liquid wax.

The white-light interferometer is designed for nanometer-scale resolution over relatively small (micron-sized) interrogation regions. As such, achieving a high density of interferometric fringes over the rather large (nearly 1 mm long) and deep (tens of microns) corneal ablation craters was difficult, sometimes resulting in incomplete
topographical information. Furthermore, in some cases irregular craters were observed,
which may be attributed to poor application of the liquid wax or to damage during
removal of the wax from the surface of the eye.

After considerable investigation, it was determined that applying the wax from a
height of about 5 mm above the surface was optimal. Dropping the wax from higher
positions could result in hardening of the leading edge of the wax droplet during descent,
leading to incomplete filling of the ablation crater and decreased accuracy. Application
of the liquid wax was more difficult and intricate for the bovine eyes as compared to the
PMMA because the wax was applied to individual ablation sites on the eyes while it was
applied to a large area inclusive of twelve ablation sites on the PMMA surface.

4.2.3 Interpretation of Depth Data and Regression Line

The PMMA regression lines were characterized by very little error in the data
points, an essentially zero intercept, and a slope (i.e., ablation rate) that was in excellent
agreement with the ablation rates based on individual crater depths and corresponding
pulse numbers. However, the bovine eye data exhibited somewhat different results.
First, there was much greater error associated with each of the data points for the bovine
eye data as compared to the PMMA data. This was attributed to previously described
issues such as incomplete filling of the craters and damage done to the wax impressions
when removing them from the eye surface.

Second, the preliminary regression line had a negative intercept and a slope
indicating an ablation rate about 20% larger than the rate determined from the individual
depth measurements. This behavior was attributed to the higher degree of scatter in the
corneal ablation data as compared to the PMMA data combined with a relatively small
number of data points. As confirmed by confocal Raman spectroscopy measurements,
the negative intercept could not be attributed to swelling of the cornea due to dabbing with moist Kim-wipes because corneal hydration remained essentially constant.

Subsequently, the final regression line was forced through the origin while maintaining a constant regression coefficient (r=0.996). This algorithm produced a regression line (as seen in Figure 4.5) consistent with the individual ablation crater data and consistent with the observed physics of the actual corneal ablation. Specifically, the ablation rate of 0.90 \( \mu m/\text{pulse} \) based on the slope of the regression line is in excellent agreement with the ablation rate of 0.87 \( \mu m/\text{pulse} \) based on the average of the individual crater depths and corresponding number of pulses. Furthermore, the lack of incubation was confirmed both visually and audibly during the experiments; hence the zero intercept is consistent with the absence of any incubation effects with excimer laser corneal tissue ablation, as reported in the literature (Pettit et al., 1991, 1995).

Although previously reported techniques for measuring ablation rates of corneal tissue have inherent limitations, as discussed in Section 1.6, it is worth noting that the corneal ablation rates reported in the current study agree favorably with previously reported values. Van Saarloos and Constable (1990) reported ablation rates approaching 0.8 \( \mu m/\text{pulse} \) at fluences of 300-400 mJ/cm\(^2\). Aron-Rosa et al. (1986) reported ablation rates ranging from 0.5-1.0 \( \mu m/\text{pulse} \) at fluences ranging from 200-1000 mJ/cm\(^2\). These values are in excellent agreement with the current ablation rate of 0.9 \( \mu m/\text{pulse} \) for an average fluence of approximately 350 mJ/cm\(^2\) and a peak fluence approaching 600 mJ/cm\(^2\).
4.3 Summary

The current technique for measuring ablation rate has been demonstrated to be both accurate and relatively precise, and is concluded to be well suited for corneal tissue measurements. More importantly, the technique is straightforward to implement, enables rapid collection of wax impressions immediately following ablation, and eliminates many of the potential difficulties and inaccuracies in measuring corneal tissue ablation rates due to changes in corneal hydration or shape.

However, it is important to recognize that, while this study implements and validates white-light interferometry as a means to obtain ablation crater profiles, the methods described herein do not constitute the only interpretation of the data. Once the crater profiles are obtained from the white-light interferometer, one could calculate ablation rates in many different ways, including the use of the average depth or the total crater volume. This study presents a peak-depth measurement of ablation rate since the deepest portion of the crater was used and the full depth of each crater was measured using the crater floor. Overall, this technique is considered to be useful for the quantitative study of corneal tissue ablation.
CHAPTER 5
COORDINATED MEASUREMENTS OF TIME-RESOLVED REFLECTANCE, CORNEAL HYDRATION AND ABLATION RATE

In Chapter 3, results were presented for bovine corneal hydration measurements that were obtained using confocal Raman spectroscopy. Ablation rate measurements using a novel method of examining wax molds of ablation craters with a white-light interferometer were presented in Chapter 4. These methods were combined, along with the time-resolved reflectance and correlation methods described in Section 2.1, into an integrated set of bovine eye experiments in an effort to determine the relationships between the corneal ablation rate, corneal hydration, laser pulse energy, and the cross-correlation function. The results of these experiments are detailed in this chapter.

5.1 Correlation of Ablation Rate and Laser Pulse Energy

Recall from Section 2.4.2 that the laser pulse energy can be calibrated using the incident waveform, which makes it possible to determine the pulse energy for every single laser pulse throughout a given set of experiments. For the bovine eye measurements presented in this chapter, the incident laser pulse waveforms for all pulses at a given site on a bovine eye were averaged together and used to obtain the average laser pulse energy for that site. The pulse-to-pulse fluctuation of energy was very small and therefore not considered for such short sequences of pulses.

The ablation rate, as measured using wax molds and white-light interferometry, is shown in Figure 5-1 as a function of laser pulse energy. There are 17 points corresponding to 17 individual ablation sites. There is a clear monotonic increase in
ablation rate with increasing laser pulse energy, which not only agrees with previous research but also intuitively makes sense. Higher laser pulse energy translates to more energy coupled into the tissue, which results in a larger ablation rate.

\[ y = 0.472 + 0.158x \]  
\[ (r = 0.755) \]

Figure 5-1. Ablation rate of bovine corneal tissue as a function of the excimer laser pulse energy. The least-squares regression line and its corresponding equation are included.

Despite the positive relationship between rate and pulse energy, the data in Figure 5-1 exhibit a great deal of scatter. The pulse energy was very accurately correlated to the integrated area of the incident waveform, meaning that the scatter in the data must be largely attributed to the ablation rate measurements. Precision issues related to the creation of wax molds of ablation craters were discussed in Chapter 4, and are considered responsible for the scatter shown in Figure 5-1. It is this scatter in the data that makes the regression coefficient \((r=0.755)\) significantly different from unity.
Figure 5-1 is a scatter plot that shows the ablation rate and laser pulse energy for each ablation site. The data were also grouped together and averaged over all sites for each eye. Depending on the availability of ablation rate data, this meant averaging different numbers of sites together. For one bovine eye, no ablation rate measurements were possible, and for another, the ablation rate could only be determined for one site. The averaged data is shown in Figure 5-2.

![Figure 5-2](image)

Figure 5-2. Bovine corneal tissue ablation rate as a function of laser pulse energy. Each data point represents the average value over all sites for a given eye, and the error bars represent ± one standard deviation.

Comparing Figures 5-1 and 5-2, there is a similar monotonic increase in ablation rate with increasing laser pulse energy. The data that was averaged for each eye, as shown in Figure 5-2, yield a regression line with a slightly higher slope and a larger regression coefficient (r=0.842). Nevertheless, both data presentations (scatter and average) display similar characteristics and show a positively correlated relationship between corneal tissue ablation rate and laser pulse energy, as expected.
5.2 Correlation of Decay Slope and Ablation Rate

As discussed in Section 1.10, one of the goals of this research was to develop a method to measure the corneal tissue ablation rate in real time that can be used in clinical systems to provide feedback. The cross-correlation function, using the incident and reflected pulse waveforms, was considered to be the prime candidate. After a great deal of effort, it was determined that the best metric that can be extracted from the cross-correlation function is the decay slope. This is the slope of the middle, decaying portion of the function, as detailed in Section 2.1.2.

For a given site, the waveforms corresponding to pulses 2 through 20 were averaged together to obtain one incident and one reflected waveform for that site. The cross-correlation function was then calculated and the decay slope determined. The relationship between the decay slope and the tissue ablation rate is shown in Figure 5-3.

![Figure 5-3. Decay slope of cross-correlation function as a function of bovine corneal tissue ablation rate. The least-squares regression line and its corresponding equation are included.](image-url)
Clearly, there is a monotonic linear increase in the decay slope with increasing ablation rate. The scatter is attributed again to the ablation rate measurements, and is considered largely responsible for the departure of the regression coefficient \((r=0.744)\) from unity. Just as before, the ablation rates and decay slopes were averaged together, wherever data were available, for all sites on a given eye. Therefore, the data are also reported in this manner, with one average ablation rate and one average decay slope for each eye. The results are shown in Figure 5-4.

![Figure 5-4. Cross-correlation function decay slope as a function of ablation rate. Each data point represents the average value over all sites for a given eye, and the error bars represent \(\pm\) one standard deviation.](image)

Comparing Figures 5-3 and 5-4, the relationship between decay slope and ablation rate is similar. The data that was averaged for each eye, as shown in Figure 5-4, have a regression line with a higher slope and a larger regression coefficient \((r=0.884)\). Nevertheless, both data presentations (scatter and average) display similar characteristics.
and show a positively correlated relationship between corneal tissue ablation rate and the
decay slope of the cross-correlation function.

It is an extremely important result that there is a distinct relationship between the
cross-correlation decay slope and the ablation rate. This means that there is a strong
possibility that the cross-correlation function can be used to provide ablation rate
feedback in clinical refractive surgery systems. It is noteworthy that the cross-correlation
function can be obtained passively without affecting clinical systems or surgical
outcomes. All that is required is the installation of two photodetectors to collect
reference (incident) and reflected excimer laser pulses.

5.3 Correlation of Ablation Rate and Corneal Hydration

It has been asserted in previous research, as detailed in Section 1.7, that the ablation
rate of corneal tissue is closely coupled to corneal hydration. However, the nature of this
relationship remains unclear, and conflicting results have been reported. One of the goals
of this work was to clarify this issue.

Recall from Section 2.2 that corneal hydration was measured using confocal
Raman spectroscopy and was quantified by the ratio of the integrated O-H Raman band
(3100-3700 cm\(^{-1}\)) to the integrated C-H Raman band (~2900 cm\(^{-1}\)). Figure 5-5 shows the
measured corneal tissue ablation rate as a function of corneal hydration, as measured by
the O-H/C-H Raman ratio. The data in Figure 5-5 all reflect approximately the same
energy to eliminate pulse energy effects. It is only useful to examine data taken at the
same energy to determine what, if any, relationship exists between ablation rate and
hydration. Consideration of data at largely different energy levels may introduce trends
or features that are only a result of the variation in energy.
Figure 5-5. Bovine corneal tissue ablation rate as a function of the Raman OH/CH ratio, which serves as a metric for corneal hydration. The regression line and its corresponding equation are included.

Recall from Section 2.4.1 that three different nominal laser energy levels were investigated. Six of the eight bovine eyes were exposed to nominal laser energy of 3.4 mJ/pulse. However, fluctuations in the laser energy over time were unavoidable. All of the energy values for these six eyes (18 total sites) were averaged together and a standard deviation was assigned. Ablation rate and corneal hydration data were only included if the laser pulse energy was within plus or minus one standard deviation of the average.

Once the inclusion criteria were established, only seven data points were left for consideration. With so few data points, it is difficult to derive any meaningful conclusions or infer any definite relationships. According to Figure 5-5, there appears to be a positive correlation between the tissue ablation rate and corneal hydration. In other words, experimental evidence suggests that ablation rate increases with increasing
corneal hydration. This relationship is discussed in greater detail in Chapter 7 as it pertains to the development of a corneal tissue ablation model.

5.4 Examination of Individual Laser Pulses

All of the results reported above were average values in some form or another. Ablation rate measurements, decay slopes, hydration measurements, and laser pulse energy values were averaged first for each ablation site, and then for all sites on each bovine eye. It is likely that any implementation of the cross-correlation function in a clinical system would involve taking measurements and calculating average values over at least several laser pulses to adjust treatment algorithms for the true ablation rate.

However, it is worth examining the behavior of the decay slope for each individual laser pulse over the course of treatment at a given site. Figure 5-6 shows the decay slope as a function of laser pulse number for the three different laser energy levels that were used to ablate the bovine eyes. The three sequences shown correspond to three different eyes and, more specifically, the first site ablated on each of these eyes. Although the results shown in Figure 5-6 represent only 3 of the 24 total ablation sites, they are typical of the results found throughout the experiments. Figure 5-7 is simply a closer view of the same results shown in Figure 5-6.

Note that the three laser energy levels shown in Figures 5-6 and 5-7 are approximately 3.1, 3.7, and 4.4 mJ/pulse. These are the average laser pulse energy values that were actually measured for each of the three different levels. In Section 2.4.1, it was stated that bovine eyes were treated using nominal laser energy values of 2.6, 3.4, and 4.1 mJ/pulse. These were the intended values, while the values appearing in Figures 5-6 and 5-7 reflect the true averages of the experimentally measured values for each of the nominal laser energy levels.
Figure 5-6. Pulse-by-pulse progression of cross-correlation decay slope for three different laser energy levels. Each decay slope progression represents laser treatment at a single ablation site. Dashed lines represent the average values of the decay slopes at each energy level.

Figure 5-7. Closer view of the results shown in Figure 5-6. Dashed lines represent the average values of the decay slopes at each energy level.
A second aspect to note is that for all three laser energy levels, the decay slopes for each individual laser pulse are for the most part tightly distributed about the corresponding average values. There is much more scatter in the values shown for the lowest energy level (~3.1 mJ/pulse), which is likely a result of the fact that the laser energy itself becomes increasingly variable as the energy level decreases. It should also be noted that there appears to be a trend at the lowest energy level. The decay slope is above the average value until the halfway point of the treatment, after which it dips below the average value and continues to decrease. This is an interesting observation that does not have any clear explanation. It is not a result of any systematic trend in the energy fluctuation, because the laser pulse energy was confirmed to be randomly scattered about the average value throughout the treatment.

A third observation concerning Figures 5-6 and 5-7 is that there is very little overlap in the values of the decay slopes for different laser energy levels. There is a clear separation between the decay slope values between the highest energy level (~4.4 mJ/pulse) and the intermediate energy level (~3.7 mJ/pulse). The separation is also marked between the intermediate and lowest energy levels, although there is some overlap. Again, this is likely due to increasing variability in pulse energy at the lower values. Nevertheless, it appears that data taken from individual laser pulses, in addition to data that is averaged over a sequence of laser pulses, may be useful in predicting corneal tissue ablation rates.

5.5 Cross-Correlation Using an Average Incident Waveform

For all of the data that has already been presented in this chapter, the cross-correlation functions were calculated based on the paired incident and reflected waveforms from a given ablation site. It is also useful to explore the necessity of
recording incident waveforms for every laser pulse to pair with the reflected waveforms. Perhaps for a clinical procedure where the laser energy is generally very stable, the incident waveform might be sufficiently stable such that one average incident waveform could be used throughout a particular treatment and all of the reflected waveforms could be paired with the same incident waveform to create cross-correlation functions.

The average incident waveform from the very first site ablated on the first bovine eye was used to generate cross-correlation functions for all of the ablation sites. Decay slopes were then calculated from the cross-correlation functions and compared with those that were previously reported in this chapter. The results of this exercise are shown in Figure 5-8.

Figure 5-8. Comparison of two methods for determining the decay slope of the cross-correlation function. The y-axis represents the value of the decay slope determined by the application of a single, universal incident waveform combined with reflected waveforms from each site. The x-axis represents the value of the decay slope determined by the normal method, using paired incident and reflected waveforms from each ablation site.
The dashed, diagonal line in Figure 5-8 represents the ideal result, where there is no difference between the decay slopes determined by the two different methods. It is clear that, at intermediate energies, the two methods result in little or no difference. However, at low energies, the use of a representative incident waveform overestimates the decay slope; at high energies, the use of a representative incident waveform underestimates the decay slope. This is a significant result, when considering the fact that the incident waveform that was universally applied was recorded at the intermediate energy level. The logical conclusion is that the shape of the incident waveform, although it appears to remain constant regardless of energy, actually changes as the laser pulse energy changes. The change in shape is very likely subtle, but is apparently magnified when the waveforms are incorporated into the cross-correlation functions. This explains why the results using a single, universal incident waveform match the original results at intermediate energy, while the results systematically diverge at significantly higher or lower energy. Nevertheless, the results indicate that it might be possible to use a single, average incident waveform if the laser energy remains relatively constant.

5.6 Summary

As expected, a positive correlation between the corneal tissue ablation rate and the laser pulse energy was confirmed. Experimental evidence has shown that as laser pulse energy increases, ablation rate also increases for the energy range that was investigated. Prior research has shown that this trend holds only up to a certain point, beyond which the ablation rate tends to level off. This plateau effect, however, only occurs well above what is considered clinically relevant. For clinically relevant laser energy or fluence, as shown in this work, the ablation rate increases steadily with laser energy.
A very important result of this work was the demonstrated positive correlation between the tissue ablation rate and the decay slope of the cross-correlation function. This finding suggests that it might be possible to implement the use of the cross-correlation function, with the decay slope as the key metric, to provide ablation rate feedback in clinical systems. Further work is necessary to determine exactly how the method should be integrated into clinical laser systems, but it is encouraging that there is a definite linear relationship between the ablation rate and the decay slope. Of further significance is the fact that the measurements necessary to obtain the decay slope are passively obtained.

Finally, a positive correlation between the tissue ablation rate and corneal hydration was demonstrated, albeit for a limited set of data. Experimental evidence suggests that ablation rate increases as corneal hydration increases, although no definite conclusions were reached about this relationship. To further elucidate the effect of corneal hydration, future work should include a larger set of measurements of corneal hydration and tissue ablation rate for constant, clinically relevant laser pulse energy. Chapter 7 of this dissertation presents a novel corneal tissue ablation model and further discusses the effect of corneal hydration on the ablation rate in a theoretical framework.
It is possible that the apparent transient decrease in reflectivity during an ablating laser pulse, as discussed previously, as well as the variation in ablation rate with hydration, is a result of water chemistry. Specifically, it is possible that water in the cornea directly absorbs at least a portion of the excimer laser light and that this effect is stronger with increasing hydration. Alternatively, the peptide bonds in collagen might in fact be the primary chromophores for 193-nm excimer laser light. To date, no definitive studies support either claim, although collagen, with its high absorption coefficient as compared to water, appears to play an important role. If one considers dynamic changes in the absorptive properties of corneal tissue, the overall picture becomes even more complicated. In this chapter, results are presented of various transmission and ablation experiments that together are intended to elucidate the roles of corneal tissue constituents, both individually and in concert, in the absorption of excimer laser light.

6.1 Water Film Transmission Measurements

6.1.1 Results

Recall from Section 2.5.1 that the transmission of excimer laser light was measured as a function of laser energy through both deionized and distilled water. Since the transmission was measured through a thin water film on a quartz flat, it was necessary to first determine the transmission through the quartz flat itself. The ratio of the transmitted signal (integrated pulse) to the incident signal (integrated pulse) for a quartz flat is shown in Figure 6-1 as a function of laser energy. Error bars are not shown because each point
is an individually measured data point rather than an average. A quadratic curve fit (r=0.987) is shown, and the equation for the curve is \( y=-0.00874x^2+0.120x+0.416 \).

![Figure 6-1. Ratio of transmitted to incident signal for a plain quartz flat as a function of laser energy, where each data point represents an individual measurement. The values used to obtain the ratio were the total integrated incident and transmitted waveforms.](image)

To determine whether the quadratic relationship in Figure 6-1 was due to the quartz itself or due to the surroundings, the same experiment was done for air with no quartz flat present. The ratio of the transmitted signal to the incident signal through quiescent air is shown in Figure 6-2 as a function of laser energy using individual data points rather than averages. Apparently, some type of photo-bleaching is present along the excimer beam path. The primary focusing lens has a focal length of approximately 168 cm (~66 inches) and the beam path is approximately 137 cm (~54 inches) longer along the transmission path as compared to the incident path. Hence the longer path and considerably higher fluence (because the laser reaches its focal point along the transmission path) of the
transmission beam is consistent with pronounced photo-activity along the transmitted beam path only.

Figure 6-2. Ratio of transmitted to incident signal for quiescent air as a function of laser energy, where each data point represents an individual measurement. The integrated incident and transmitted waveforms were used to obtain the ratio.

Clearly, the transmission ratio (transmitted/incident signal) shows a similar trend with respect to laser energy for both air and the quartz flat. Therefore, the quartz flat measurements were corrected by dividing by the transmission ratio of air at the appropriate energy. The curve fit ($r=0.994$) for the air data was also quadratic, with equation $y=-0.0123x^2+0.161x+0.428$. Each individual quartz transmission data point was corrected by dividing by the air transmission signal as determined by the above equation at the correct energy, as well as accounting for Fresnel losses. The corrected transmission of the quartz flat is shown as a function of laser energy in Figure 6-3, with an essentially flat trend and an average transmission value of $0.974 \pm 0.025$ (2.56\% relative standard deviation, rsd).
Figure 6-3. Transmission through a quartz flat, corrected for air transmission and Fresnel losses, as a function of laser energy. Each data point represents an individual measurement, therefore no error bars are shown.

Measured on two separate days, the small-signal absorption coefficient of deionized water was determined to be 0.15 cm\(^{-1}\), a value that agrees well with the published data summarized in Figure 1-4. The transmission of laser light through deionized water, which was the true transmission corrected for the quartz flat and Fresnel losses, was then determined as a function of laser energy. Assuming that the small-signal absorption coefficient was relevant at the lowest energy for which transmission was measured, the absorption coefficient of deionized water was then determined as a function of laser energy as described in Section 2.5.1. The same exercise was done for distilled water, including the determination of the corrected transmission and the calculation of the absorption coefficient as a function of laser energy using the small-signal absorption coefficient of deionized water as the reference value. Results for both deionized and distilled water are shown in Figures 6-4 and 6-5, respectively.
Figure 6-4. Absorption coefficient of deionized water as a function of laser energy. Each point is an average value \((N=3)\) and the error bars for both laser energy and absorption coefficient represent \(\pm\) one standard deviation.

Figure 6-5. Absorption coefficient of distilled water as a function of laser energy. Each point is an average value \((N=3)\) and the error bars for both laser energy and absorption coefficient represent \(\pm\) one standard deviation.
6.1.2 Interpretation of Results

The small-signal coefficient was also measured for distilled water, but two different experiments produced two vastly different values, most likely due to differences in the distilled water composition. This might also explain the relatively large error bars for the absorption coefficient of distilled water as compared to deionized water (see Figures 6-4 and 6-5). Therefore, the value of the small-signal absorption coefficient for deionized water was used for both deionized and distilled water, and was assumed to be valid at the lowest energy level (~0.75 mJ/pulse).

That the absorption coefficient of water, both deionized and distilled, remained relatively constant over a wide range of laser fluences is an important result. As mentioned in Section 1.8.1, prior research has shown that superheated water is a much stronger absorber of 193-nm light than normal liquid water (Staveteig & Walsh, 1996). However, it has been reported that absorption of infrared laser light in a water cell is significantly enhanced only when the laser fluence exceeds 10 J/cm² (Niemz, 1996). The current research, which agrees with the latter, suggests that the excimer laser, even at fluences significantly exceeding clinical relevance, does not superheat the water or initiate any other perturbation to the optical properties (e.g., plasma initiation), hence there is no enhancement to the baseline water absorption.

6.2 Transmission Through Solutions of Collagen and Amino Acids

6.2.1 Results

Recall from Section 2.5.2 that transmission of 193-nm excimer laser light was measured through solutions containing collagen, which contained amino acids joined by peptide bonds, and solutions containing isolated amino acids with no peptide bonds.
Furthermore, transmission was measured through deionized water and acetic acid to provide reference values for the correction of these transmission measurements.

The average transmission measured through the cell containing only deionized water after correcting for Fresnel losses was calculated as $0.98 \pm 0.01$ (0.8% rsd). Using this value and the known 0.167-cm pathlength, the Beer-Lambert law was used to calculate the absorption coefficient of the water, yielding a value of $0.15 \pm 0.05$ cm$^{-1}$ (31.5% rsd), which is in excellent agreement with the value reported in Section 6.1.1 as well as the published data summarized in Figure 1-4. The rather large relative error is reflective of the near unity transmission value for this pathlength and the logarithmic nature of the Beer-Lambert law.

Using the average absorption coefficient for water, and the measured transmission values of water and acetic acid, the absorption coefficient of acetic acid was determined to be $30.1 \pm 0.3$ cm$^{-1}$ (1% rsd) for the 0.5 N solution. The near unity value of water transmission and the corresponding large relative error of the water absorption coefficient do not adversely impact the precision of the acetic acid absorption coefficient due to the nearly 200-fold increase in acetic acid absorption as compared to water.

Using the absorption coefficients of water and acetic acid, transmission measurements for dissolved collagen solutions of varying concentrations were converted to absorption coefficients. Furthermore, the mass concentrations were converted to peptide bond number densities using Eq. 2-9 in Section 2.5.2. Using similar procedures, the recorded transmission measurements of the 1:1:1 glycine, proline, and hydroxyproline amino acid solutions were converted to absorption coefficients. The results, for both the collagen and amino acid solutions, are summarized in Figures 6-6 and 6-7.
Figure 6-6. Collagen absorption coefficient as a function of the number density of peptide bonds contained in the collagen. Error bars represent ± one standard deviation and the solid line represents the least-squares fit line.

Figure 6-7. Absorption coefficients of collagen and amino acids as a function of the mass concentration of the solutions. Error bars represent ± one standard deviation and the solid lines represent the least-squares fit lines.
6.2.2 Interpreting the Results

The absorption coefficient is the product of the chromophore number density and the chromophore absorption cross-section. Hence the slope of the plot in Figure 6-6 yields the absorption cross-section of the amino acid unit, which is shown to be approximately $1.19 \times 10^{-17}$ cm$^2$. An amino acid unit consists of a single amino acid and a peptide bond, both of which are chromophores for 193-nm excimer laser light. The relative absorptive contribution of the amino acids themselves is shown in Figure 6-7. Results are plotted as a function of solution mass concentration because the peptide bond number density has no relevance for the isolated amino acid solutions.

Previous studies dating to the 1960s have investigated the absorption of ultraviolet light by proteins, which include amino acids and the peptide bonds that join them, as well as by isolated amino acids that contain no peptide bonds (Wetlaufer, 1962). However, these studies have generally concentrated on radiation in the near-UV range of 240-290 nm, since 193-nm radiation was essentially irrelevant when the studies were performed. Hence an additional goal of the current study was to investigate the absorptive properties of isolated amino acids (i.e., no peptide bonding) for 193-nm radiation to quantify the role of the peptide bond as the primary chromophore.

As demonstrated in Figure 6-7, the amino acids themselves have an absorption cross-section that is approximately 4% of the value of the absorption cross-section for collagen. This was determined using the slopes of the regression lines for amino acids and collagen, which are 3.01 and 80.3, respectively. Based on these results, the actual absorption cross-section of the collagen peptide bond is approximately $1.14 \times 10^{-17}$ cm$^2$, or 4% lower than the value reported above, and the average absorption cross-section of an amino acid in collagen is approximately $4.74 \times 10^{-19}$ cm$^2$. These results have
effectively separated collagen into its constituent chromophores—the peptide bonds, which account for nearly all of the absorption, and the amino acids. The total effective absorption cross-section for collagen is then the sum of these two, or $1.19 \times 10^{-17}$ cm$^2$ per amino acid unit (i.e., amino acid plus peptide bond).

6.2.3 Applying the Results to Corneal Tissue

The collagen absorption cross-sections measured in the current study can be used to estimate the absorption coefficient of corneal tissue. Corneal tissue was assumed to be composed of 20% collagen and 80% water and the tissue density was assumed to be equal to that of water (1 g/cm$^3$). Referring to Eq. 2-9, the peptide bond density was determined to be $6.77 \times 10^{21}$ cm$^{-3}$. Therefore, the equivalent corneal tissue absorption coefficient was calculated from the following relation:

$$\alpha_{tissue} = (0.2)(1.19 \times 10^{-17} \text{ cm}^2)(6.77 \times 10^{21} \text{ cm}^{-3})$$  

Using Eq. 6-1, the absorption coefficient of corneal tissue was determined to be approximately 16,000 cm$^{-1}$. The effective absorption cross-section of a water molecule is $4.5 \times 10^{-23}$ cm$^2$ based on the measured absorption coefficient (0.15 cm$^{-1}$), making the contribution of water to the tissue absorption coefficient negligible.

It is noted that this value (16,000 cm$^{-1}$) is based solely on the contribution of collagen, as quantified in isolated solutions. However, actual corneal tissue will undoubtedly contain additional conformational changes, and may contain other chromophores such as glycosaminoglycans, as suggested earlier (Puliafito et al., 1985). Nonetheless, research has supported the argument that the absorption coefficient of corneal tissue is on the order of 20,000 cm$^{-1}$ or more. In fact, Pettit and Ediger (1996)
used molar absorption coefficient data to estimate an equivalent corneal tissue absorption coefficient of 20,000 cm$^{-1}$, which is in very good agreement with the current value.

Notwithstanding this agreement, a key issue that must be considered in the context of corneal absorption coefficients relevant to excimer laser ablation is the nature of a static value as applied over a significant range of laser energy, including under ablative conditions. The value calculated in this study (16,000 cm$^{-1}$) is truly a “small-signal” (i.e., sub-ablative condition) corneal absorption coefficient, which is likely to be enhanced as a result of laser-tissue interactions realized with significantly higher pulse energy, notably within the ablation regime.

Prior research has documented evidence of a dynamic enhancement in tissue absorption and reflection properties during 193-nm laser ablation. However, with previously reported corneal tissue absorption coefficients as low as 2400 cm$^{-1}$, it was difficult to reconcile the order of magnitude increase in absorption necessary to explain ablation rate data. Based on the current measurements of collagen absorption properties, the predicted corneal absorption coefficient of 16,000 cm$^{-1}$ necessitates only modest dynamic enhancement (not order of magnitude) for consistency with reported ablation rate data in the context of the Beer-Lambert model. The dynamic behavior of corneal absorption is further discussed later in this dissertation.

### 6.3 Thin Collagen Film Transmission Measurements

#### 6.3.1 Results

Recall from Section 2.5.3 that transmission was measured, for varying laser energy, through thin collagen films that were created by drying dissolved collagen solutions on quartz flats. Specifically, the ablation was carried out in a repeating, alternating pattern
of four rows on each of three films, such that there were a total of twelve ablation sites corresponding to each nominal laser energy level.

For each ablation site, following the first few laser pulses, the pulse-to-pulse normalized transmission was observed to increase monotonically to the asymptotic value of unity. The pulse-to-pulse progression of the normalized transmission is presented in Figure 6-8 for laser pulse energy of approximately 0.9 mJ/pulse, as averaged over twelve different ablation sites. The average film thickness, as measured by white-light interferometry, was $3.2 \pm 0.5 \mu m$ (15% rsd). The full film thickness was sufficiently large to initially render the film opaque to the incident laser pulse, hence the first few laser pulses resulted in zero transmission. Subsequent laser pulses steadily ablated through the full thickness of the collagen film.

![Figure 6-8](image)

Figure 6-8. Pulse-to-pulse progression of the transmission of 193-nm laser light through thin collagen films, normalized by the steady-state value reached after complete film perforation. Error bars represent one full standard deviation ($N=12$).
The pulse-to-pulse progression of the transmission data was then used to calculate an equivalent ablation rate for each recorded laser pulse energy. The transmission profiles corresponding to the lowest energy (~0.9 mJ/pulse) and the highest energy (~3.8 mJ/pulse) are plotted in Figure 6-9. The dotted lines indicate the points at which the normalized transmission values have reached 50% of their steady-state values for the different energy levels. As expected, 50% transmission is reached sooner for higher energy than for lower energy.

![Figure 6-9](image)

**Figure 6-9.** Transmission profiles, each averaged over twelve ablation sites, corresponding to laser energy of 0.9 and 3.8 mJ/pulse. Solid lines represent smooth curve fits to the data. Dashed lines indicate the 50% transmission point and the corresponding number of laser pulses for both data sets.

The point of 50% transmission and the corresponding number of laser pulses to reach this value were used to define the characteristic ablation rate. Specifically, for each laser pulse energy, the ablation rate was calculated as the average film thickness (3.2 µm) divided by the number of laser pulses required to reach 50% normalized transmission.
The measured ablation rate as a function of pulse energy is shown in Figure 6-10 for the collagen films. The error bars reflect the average error in the number of pulses to achieve 50% transmission, as calculated using the error bars in the normalized transmission profiles (see Figure 6-8). Over the nominal range of pulse energy from 1 to 4 mJ/pulse, the collagen film ablation rates were found to vary from about 0.35 µm/pulse to greater than 0.45 µm/pulse. To avoid ambiguity associated with the laser beam profile, the ablation rate data are reported as a function of total pulse energy rather than fluence.

![Figure 6-10](image-url)

Figure 6-10. Ablation rate of thin collagen films as a function of laser pulse energy. Error bars represent ± one standard deviation, based on the average error in the number of laser pulses required to achieve 50% transmission.

### 6.3.2 Predicting Ablation Rates Using the Beer-Lambert Law

To explore the role of collagen absorption characteristics, namely the peptide bond absorption cross-section, in excimer laser ablation, the ablation rates of the collagen films were modeled using the Beer-Lambert blow-off model. The blow-off model, as discussed in Section 1.9, is described as follows:
\[ d_{\text{ablation}} = \frac{1}{\alpha} \ln \left( \frac{I_0}{I_{th}} \right) \]  

This model requires an effective absorption coefficient of a collagen film and an ablation threshold energy to predict the ablation depth as a function of pulse energy. The ablation threshold was experimentally determined for the same collagen films, by incrementally decreasing the laser pulse energy to determine the value at which the collagen film remained intact, regardless of the number of laser pulses. Lack of ablation was verified by the absence of transmission through the film for hundreds of laser pulses. Based on repetitive trials, the average ablation threshold was determined to be 0.053 mJ/pulse. This experimental value was then used for the Beer-Lambert blow-off model. Using the measured full beam area, this corresponds to an average threshold fluence of ~10 mJ/cm\(^2\), which is in the range of threshold values expected for tissue and collagen. The peak fluence is expected to be two to three times greater based on the actual beam profile.

Because absorption coefficients are additive, the absorption coefficient of a collagen film was calculated as the sum of the absorption contribution of the peptide bonds and the contribution of the amino acids in the collagen. The peptide bond contribution was equal to the product of the peptide bond absorption cross-section and the peptide bond number density, and the amino acid contribution was equal to the product of the amino acid absorption cross-section and the amino acid number density.

For the collagen structure, the peptide bond number density is assumed equal to the amino acid number density, although the total number of peptide bonds is three less than the total number of amino acids due to the triple stranded structure and the absence of a peptide bond at the end of each chain. The peptide bond number density in a collagen film is equal to the absolute number of peptide bonds divided by the total volume of the
film. The absolute number of peptide bonds was calculated as the product of the peptide bond number density in the solution used to prepare the film (see Eq. 2-9) and the volume deposited to form the film (5 mL).

The surface area of a dry collagen film was not measured directly, due to the transparent nature of the films, the approximate 3-μm thickness, and the irregular contour of the edges. However, careful examination in reflected light revealed that the films were certainly smaller than the entire area of the quartz flats (50 mm diameter), but were considered larger than the area defined by the inner half diameter (i.e., 25 mm diameter) of a quartz flat. Working within these two limiting areas, the total surface area of a dry collagen film was estimated to be two-thirds of the total available area of the quartz flat, hence the surface area was equal to 13.5 cm². Using the measured film thickness, the average total volume of a collagen film was therefore estimated to equal 0.004 cm³.

Each collagen film was prepared with 5 mL of collagen solution at a concentration of 1 mg/ml, which when combined with Eq. 2-9 and the total volume of a thin film, yields a peptide bond density of about $7.8 \times 10^{21}$ cm⁻³. Using the respective absorption cross-sections of the peptide bonds and amino acids yields a collagen film absorption coefficient of approximately 91,900 cm⁻¹.

Finally, using the measured ablation threshold of 0.053 mJ/pulse, and the calculated absorption coefficient of 91,900 cm⁻¹, the Beer-Lambert blow-off model (see Eq. 6-2) was applied to predict ablation rates over the range of laser pulse energy corresponding to the thin film ablation study. A comparison of the experimentally determined ablation rates and the results from the Beer-Lambert blow-off model described above is shown in Figure 6-11.
It is noted that because Eq. 6-2 utilizes the *ratio* of incident and threshold intensities, identical results are obtained whether using absolute pulse energy or laser fluence. The agreement between the experimental data and the results of the Beer-Lambert model is considered very good, given the independent measurement of the ablation threshold, the direct calculation of the absorption coefficient from independently measured absorption cross-sections, and the definition of the experimental ablation rate as based on the 50% transmission point of the pulse-to-pulse transmission profiles. A point-to-point comparison of the predicted ablation rates and the experimental data over all data points yields an average difference of 3.8%.

![Figure 6-11](image_url)

**Figure 6-11.** Comparison of the Beer-Lambert blow-off model with experimentally measured ablation rates for thin collagen films. Experimental results are shown as individual data points, with error bars representing ± one standard deviation. Rates predicted by the model are represented by the solid curve.
6.4 Summary

Water film transmission measurements demonstrated that the absorption coefficient of water remains very small (~ 0.15 cm$^{-1}$) regardless of laser energy, even at levels far exceeding clinical relevance. The corresponding absorption cross-section of a water molecule is approximately $4.5 \times 10^{-23}$ cm$^2$, which is negligible compared to the experimentally determined effective cross-section of collagen, which is equal to $1.19 \times 10^{-17}$ cm$^2$ per amino acid unit. Hence, any laser-induced photochemistry or dynamic enhancement of the absorption cross-section of water is unlikely to be solely a direct result of excimer laser interactions with water. This does not eliminate, however, the possibility of water-related photochemistry in actual corneal tissue under ablative conditions. Furthermore, it has been demonstrated that the peptide bond between adjoining amino acids is responsible for approximately 96% of the absorption of collagen, which yields an absorption cross-section of $1.14 \times 10^{-17}$ cm$^2$ per peptide bond. The remaining 4% of collagen absorption is due to the amino acids themselves, corresponding to an average amino acid absorption cross-section of $4.74 \times 10^{-19}$ cm$^2$.

This study is believed to be the first to directly quantify the peptide bond as the primary collagen chromophore for 193-nm laser radiation. Previous research suggests that in general peptide bond absorption may be dependent on the actual conformation of the overall protein (Wetlaufer, 1962), but this effect was not considered in these experiments. Nonetheless, the conformation of the collagen used in the current experiments is considered representative of collagen present in corneal tissue.

For thin collagen films, a Beer-Lambert blow-off model was formulated using the measured peptide bond and amino acid absorption cross-sections, the estimated collagen film bond density, and the measured ablation threshold. The corresponding absorption
The coefficient of the dry collagen films was calculated as approximately $91,900 \text{ cm}^{-1}$, which yielded very good agreement between the Beer-Lambert model and the experimental ablation rate data. Such agreement is considered strong evidence to support the measured absorption cross-sections of collagen and more importantly, the role of the peptide bond in collagen ablation. It is noted that the agreement between the model and experiments is based on a *static* absorption coefficient, which is believed appropriate for the thin, *dry* collagen films. This is consistent with previous work with corneal tissue that shows tissue hydration plays a role in the dynamic changes in tissue optical properties under ablative conditions (Ediger et al., 1993a, 1993b).

Measurement of the actual constituent absorption cross-sections enables calculation of the equivalent absorption coefficient of corneal tissue based on a tissue composition of 20% collagen and 80% water. The result is an absorption coefficient of $16,000 \text{ cm}^{-1}$ for corneal tissue at 193 nm. This value is much larger than early measurements of 2400 to 2700 cm$^{-1}$ (Lembares et al., 1997; Puliafito et al., 1985; Puliafito et al., 1987b), but on the same order of magnitude as more recent estimates in the range 20,000 to 40,000 cm$^{-1}$ (Bor et al., 1993; Pettit & Ediger, 1996; Yablon et al., 1999).

While measured at subablative fluences, these higher values (i.e., 20,000-40,000 cm$^{-1}$) can be used in a Beer-Lambert blow-off model to predict ablation rates that are consistent with experimentally measured rates for a range of data, while the lower values (i.e., 2400-2700 cm$^{-1}$) grossly overestimate the rate of corneal tissue ablation (Pettit & Ediger, 1996). However, both cases should be considered in the context of ablation-induced laser-tissue interactions, namely dynamic changes in optical properties manifested as transient changes in tissue absorptivity and reflectivity.
The reconciliation of observed ablation rate data, observed transient changes in tissue property, and the range of corneal tissue absorption coefficients as reported in the literature may be explained by: (i) an extremely high corneal tissue absorption coefficient (~30,000 cm\(^{-1}\)) that remains essentially static such that tissue ablation is well-described by a Beer-Lambert blow-off model, or (ii) tissue ablation effects producing an order of magnitude enhancement in the absorption coefficient from the small-signal value (i.e., increase from \(~3000\) to \(~30,000\) cm\(^{-1}\)) as a result of laser-tissue interactions. Neither explanation is completely satisfactory given that the ablation process likely causes a dynamic enhancement in tissue absorption in view of the large body of experimental evidence. However, an order of magnitude enhancement is difficult to explain.

The current study supports a view that is between these two limiting cases, namely a small-signal absorption coefficient of corneal tissue equal to about 16,000 cm\(^{-1}\) for 193-nm radiation, as based on the measured peptide bond and amino acid absorption cross-sections. Such a value is consistent with the reported range of ablation rate data assuming a modest dynamic increase of 25 to 75%. Such a degree of dynamic change is consistent with the need for some transient perturbation in tissue properties for consistency with experimental observations, while also being more acceptable than an order of magnitude change under modest ablative conditions. Overall, the current study further elucidates the role of collagen, notably the peptide bonds, in excimer laser tissue ablation, although the exact nature of these laser-tissue interactions is not sufficiently delineated. Efforts to model the ablation process and explain the ablation mechanisms based on the current research are detailed in Chapter 7 of this dissertation.
CHAPTER 7
ABLATION MECHANISMS AND MODELING

The work presented in Chapters 3, 4, and 5 included the development of tools to measure the ablation rate and relate it to clinically relevant parameters such as laser fluence and corneal hydration. A practical goal was to develop a method for real-time feedback of the corneal tissue ablation rate to improve clinical laser refractive procedures. However, as detailed in Section 1.10, this doctoral research was also devoted to gaining a better understanding of the ablation process and the mechanisms involved, as well as the development of a comprehensive ablation model. The work presented in Chapter 6 was the first step toward these more scientific goals, as the primary chromophore for 193-nm excimer laser light was identified as the peptide bond in collagen, and water itself was shown to be non-absorbing. This chapter is divided into two primary sections, with the first section including the results of emission experiments that were intended to elucidate the possible role of plasma effects in the ablation process, and the second section detailing the development of a novel ablation model.

7.1 Emission Experiments and Plasma Considerations

7.1.1 The Laser-Induced Plasma

One possible mechanism of excimer laser tissue ablation is a laser-induced plasma that leads to ablation. It is also possible that a relatively low-energy plasma is created during the ablation event, a plasma which then shields some of the incoming laser radiation and causes a decrease in reflectivity and incident energy. A laser-induced plasma is typically created in air when the laser fluence reaches ~$10^{14}$ W/cm$^2$ and optical
breakdown occurs (Niemz, 1996). Molecules in the plasma volume are dissociated into their constituent atoms, some of which are ionized, and the laser-induced plasma is characterized by an extremely high free electron density and temperature (~10,000 to 30,000 K). As the plasma cools, the energetic atoms and ions return to their ground states and eventually recombine into molecules. The return of the atoms to their ground states releases discrete amounts of energy in the form of photons. Each type of atom has characteristic energy transitions, and the release of photons with energy corresponding to these transitions is called atomic emission.

A spectrum of laser-induced plasma emission is characterized by specific, discrete atomic emission peaks or lines as well as a broad continuum emission. The relative intensities of the continuum and atomic emission processes vary temporally. The atomic emission lines that are of interest for the current work are listed in Table 7-1, which also includes the wavelength and the energy values for each transition. For the element state, “I” denotes a neutral atom while “II” denotes a singly ionized atom.

### Table 7-1. Atomic emission lines of interest.

<table>
<thead>
<tr>
<th>Element/State</th>
<th>Wavelength (nm)</th>
<th>Energy transition levels (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon I (C I)</td>
<td>247.86</td>
<td>21,648 – 61,982</td>
</tr>
<tr>
<td>Calcium I (Ca I)</td>
<td>422.67</td>
<td>0 – 23,652</td>
</tr>
<tr>
<td>Calcium II (Ca II)</td>
<td>393.37 396.85</td>
<td>0 – 25,414 0 – 25,192</td>
</tr>
<tr>
<td>Sodium I (Na I)</td>
<td>588.995 589.592</td>
<td>0 – 16,793 0 – 16,956</td>
</tr>
<tr>
<td>Hydrogen I (Hα)</td>
<td>656.28</td>
<td>82,259 – 97,492</td>
</tr>
</tbody>
</table>

With regard to plasma emission, atomic emission lines are typically very strong beginning within microseconds (µs) after plasma creation, and atomic emission as well as broadband continuum emission can be observed for at least several microseconds. It
should also be noted that the $H\alpha$ and C I emission lines at 656.28 nm and 247.86 nm, respectively, often serve as indicators of a laser-induced plasma formed in air, due to the presence of carbon dioxide and water vapor, and in tissue, due to the presence of hydrocarbon-rich proteins.

7.1.2 Emission Spectra and Experimental Results

Emission spectra were recorded during porcine eye ablation according to the procedures outlined in Section 2.6. Two laser energy levels and several spectral windows were investigated, and all spectra were corrected using calibrated broadband light sources with known irradiance distributions. The results are shown in Figures 7-1, 7-2, and 7-3, with the spectra in Figure 7-1 resulting from the combination of several spectral windows as described in Section 2.6.2. Note that, although the intensity scale units are indicated as arbitrary, the intensities for all of the spectra are on the same relative scale.

![Figure 7-1](image)

Figure 7-1. Emission spectra, after intensity correction and combination of spectral windows, from porcine eyes during ablation. The upper spectrum corresponds to laser energy of 5 mJ/pulse and the lower spectrum corresponds to laser energy of 3.5 mJ/pulse. The C I and Ca I emission lines are noted at their respective locations.
Figure 7-2. Porcine eye emission spectra recorded during ablation, with the upper and lower spectra corresponding to 5 and 3.5 mJ/pulse, respectively. The Na I emission lines are identified at their respective locations.

Figure 7-3. Porcine eye emission spectra recorded during ablation. The expected location of the 656.28-nm Hα line is noted, although this line is not present for either spectrum.
There are three observations that can be made that suggest that it is unlikely that a laser-induced plasma is formed during ablation under the current conditions. First, there is a lack of broadband continuum emission that typically characterizes a laser-induced plasma. The most prominent feature in Figure 7-1 is the broad peak centered at approximately 320 nm, which is significantly higher in intensity than the C I and Ca I atomic emission lines. This broad feature is too well defined to be continuum emission over this spectral range. Furthermore, this feature, which spans tens of nanometers, is not an atomic emission line, because atomic emission lines are generally much less than a nanometer wide. Finally, the ratio of carbon atomic emission to continuum emission does not support a laser-induced plasma, as the carbon content of a biological material would be expected to produce a significant peak-to-continuum ratio. The spectral features characteristic of a laser-induced plasma are absent in these measurements.

Second, it is clear in Figure 7-3 that there is no trace of the H\textsubscript{α} line at 656.28 nm, an atomic emission line that should be present in a laser-induced plasma spectrum when air and hydrocarbon species are present. There is no hint of this emission line even at a laser energy level of 5 mJ/pulse, which is relatively high in terms of clinical relevance. It should be noted that the two spectra in Figure 7-3, corresponding to the two incident laser pulse energies, are indistinguishable in that they have essentially the same intensity. In contrast, laser-induced plasma emission generally scales strongly with laser pulse energy.

Third, the time dependence of the observed emission was not indicative of a typical laser-induced plasma. In these experiments, no emission was observed when an additional 100 nanoseconds (ns) of delay was added before signal detection. A laser-induced plasma will generally display pronounced emission, both from atomic emission
lines and continuum, for at least microseconds when created with a nanosecond-scale laser pulse. Considering these observations, the data shown in Figures 7-1, 7-2, and 7-3 most likely represent prompt fluorescence from the rich organic matrix of corneal tissue.

The time scale of the emission detected in these experiments supports the presence of corneal tissue fluorescence rather than the creation of a laser-induced plasma. Fluorescence is a prompt phenomenon that is very strong for a short period of time and rapidly decays, as was the case in this study. Furthermore, the broad feature shown in Figure 7-1 is typical of the fluorescence observed for biological materials, which usually display spectrally broad but clearly defined bands (Prasad, 2003).

Additional evidence that favors fluorescence over a laser-induced plasma can be found in the specific spectral lines of sodium, calcium, and carbon. In Figure 7-2, the Na I emission lines are broad (a few nanometers wide) and not well resolved. Figure 7-4 shows the Na I emission lines from a laser-induced plasma using the same detection and collection system as for the current experiments.

![Figure 7-4. Laser-induced plasma emission spectrum with the two Na I emission lines properly indicated.](image-url)
The contrast between the spectra in Figure 7-2 and the spectrum in Figure 7-4 is clear. The Na I emission lines shown in Figure 7-4 are much narrower, much more intense relative to the surrounding continuum emission, and are spectrally resolved down to the baseline. The Na I emission lines shown in Figure 7-2 are comparatively broad and unresolved, and have much lower intensity with respect to the continuum emission. These contrasting characteristics suggest that the spectra shown in Figure 7-2 originate from a process other than the thermal excitation realized in a laser-induced plasma. A comparison of the two types of spectra is shown in Figure 7-5 using the low-energy ablation emission spectrum.

![Comparison between a low-energy (3.5 mJ/pulse) ablation emission spectrum (upper) and a laser-induced plasma emission spectrum (lower). The intensity scales are arbitrary and the limits were chosen to facilitate visual comparison.](image)

It has been asserted that the Na I emission lines are uncharacteristic of a laser-induced plasma. They may, in fact, be more indicative of a process such as
photofragmentation and subsequent fluorescence. Ultraviolet lasers have been known to cause dissociation of molecular compounds and create electronically excited photofragments, which fluoresce as they de-excite (Bradshaw, Rodgers & Davis, 1982; Simeonsson & Sausa, 1996). The fluorescence emission lines are often broadened due to the fact that the large amount of available energy from the ultraviolet laser can perturb the energy levels of the fragment species or atoms. Published studies, where emission was recorded following laser photofragmentation of sodium-containing aerosols, have shown Na I emission with similar characteristics as the current porcine eye fluorescence experiments (Nunez, Cavalli, Petrucci & Omenetto, 2000; Nunez & Omenetto, 2001).

Figure 7-1 displays a prominent and broad fluorescence band, but also two distinct atomic emission lines. A closer view of the Ca I emission line is shown in Figure 7-6. At lower energy (3.5 mJ/pulse) this feature is very small, and it still has relatively low intensity even at high laser energy (5 mJ/pulse). It should be noted that the spectra in Figures 7-1 and 7-6 show no trace of the Ca II emission lines at 393.37 nm and 396.85 nm. A previous study was done in which corneal hydration was measured using a laser-induced plasma method known as LIBS, or laser-induced breakdown spectroscopy (Pallikaris et al., 1998). In that study, the emission of a laser-induced plasma formed at the corneal surface was recorded, and strong calcium emission was observed. The LIBS spectra in the Pallikaris study displayed Ca I emission at 422.67 nm, as well as Ca II emission at both 393.37 nm and 396.85 nm. Furthermore, the Ca II emission lines were more intense than the Ca I emission line. Based on the experiments of Pallikaris et al. (1998), one would expect to observe Ca II atomic emission lines in the presence of Ca I emission if a laser-induced plasma is created. This is contrary to what was found in the
current emission experiments, where only weak Ca I emission was observed at 422.67 nm and no Ca II emission was observed at all.

Figure 7-6. Closer view of emission spectra shown in Figure 7-1, focusing on a spectral window centered at 410 nm. The upper and lower spectra correspond to laser energy levels of 5 and 3.5 mJ/pulse, respectively. The locations of the Ca I and Ca II emission lines are indicated, although that Ca II emission was not observed for either spectrum.

A closer view of the C I emission line is shown in Figure 7-7. The upper and lower spectra, corresponding to 5 and 3.5 mJ/pulse, respectively, are difficult to distinguish because they have similar shape and intensity. It can be seen that, even at the higher laser energy of 5 mJ/pulse, the C I emission line is relatively weak, especially compared to the large, broad fluorescence band shown in Figure 7-1. Just as with the Na I emission lines observed in the current study, the C I emission lines are not considered to be evidence of a laser-induced plasma and may in fact be due to a non-thermal process such as photofragmentation and subsequent fluorescence.
Figure 7-7. Closer view of emission spectra shown in Figure 7-1, focusing on a spectral window centered at 248 nm. The upper and lower spectra correspond to laser energy levels of 5 and 3.5 mJ/pulse, respectively and the C I (247.86 nm) emission line is identified.

7.1.3 Summary of Emission Experiments

As shown in Figure 7-1, there was a broad feature with relatively high intensity that was typical of prompt fluorescence from a biological material rather than the broad continuum emission from a laser-induced plasma. This fluorescence band was much more prominent than the C I (247.86 nm) and Ca I (422.67 nm) atomic emission lines, an observation that is not expected for a laser-induced plasma.

Figure 7-2 shows the Na I emission lines at 588.995 nm and 589.592 nm, which appear very broad and are unresolved from each other. Figure 7-5 illustrates the contrast between the sodium emission observed for these experiments and sodium emission that is typical in a laser-induced plasma, where the two emission lines are extremely narrow and well resolved down to the baseline continuum.
The H$_\alpha$ emission line at 656.28 nm was not observed, even at 5 mJ/pulse, in these experiments as shown in Figure 7-3. Both the H$_\alpha$ line and the C I line (247.86 nm) are pronounced features that are commonly observed for a laser-induced plasma in air or tissue. Furthermore, the Ca II emission lines at 393.37 nm and 396.85 nm were not observed in these experiments. Prior research has shown that Ca II emission is observed in conjunction with Ca I emission for a laser-induced plasma, and that the Ca II emission is typically more prominent than the Ca I emission. Despite the presence of the Ca I emission line in these experiments, no trace of the Ca II emission lines was found.

Finally, no measurable emission was present in these experiments when the signal detection was shifted an additional 100 ns after the excimer laser pulse. For a typical laser-induced plasma, both continuum and atomic emission are observed for at least microseconds after the laser pulse. All of this evidence strongly supports the conclusion that the observed emission was prompt fluorescence due to direct excimer laser excitation, rather than emission from a laser-induced plasma formed in or near the tissue.

It is proposed that there are two fluorescence-related processes that result from an ablation event. Specifically, the broadband emission, including both the low-intensity continuum and the broad peak centered at 310 nm, is a result of prompt fluorescence from tissue surrounding (i.e., below and adjacent to) the ablation zone. This tissue absorbs a portion of the 193-nm excimer laser light that is not sufficient to cause ablation; hence the tissue remains intact to fluoresce. If one considers laser energy to penetrate into the tissue and ablate until it is attenuated to a threshold value, then one might expect the remaining, fluorescing tissue to be exposed to a constant fluence regardless of the incident value. Therefore, the fluorescence signal is nominally independent of the
incident laser fluence. Superimposed on the broadband emission are discrete atomic emission lines that are likely a result of a photofragmentation-fluorescence or a similar non-thermal process. This process occurs within the ablated mass, and is therefore dependent on the incident laser fluence. Overall, this theory might explain the fact that the atomic emission lines appear to scale proportionately with laser energy, while the broadband emission does not.

7.2 Ablation Modeling

To date, efforts to model the ablation process and mechanisms, as well as efforts to predict ablation rates have proven unsatisfactory. The Beer-Lambert blow-off model can be used to predict the ablation rate of thin, dry collagen films with reasonable accuracy, as shown in Section 6.3.2. However, this model is based on a static absorption coefficient and does not include effects such as dynamic enhancement of absorption during ablation of corneal tissue. Ablation rates of corneal tissue from different species, including bovine, porcine, and human, have been reported in the literature and are shown in Figure 7-8 as a function of the ablating laser fluence (Aron-Rosa et al., 1986; Berns et al., 1999; Campos et al., 1993; Fantes & Waring, 1989; Huebscher et al., 1996; Kitai et al., 1991; Krueger & Trokel, 1985; Puliafito et al., 1987b; Van Saarloos & Constable, 1990).

The Beer-Lambert blow-off model, assuming a reasonable ablation threshold fluence of 50 mJ/cm², was used to predict the corneal tissue ablation rate as a function of laser fluence, the results of which are also shown in Figure 7-8. The upper of the two model curves was produced using a tissue absorption coefficient of 16,000 cm⁻¹, the value that was obtained in Section 6.2.3 using the measured fundamental absorption cross-section of collagen reported in Section 6.2.2. The lower model curve was produced
using a tissue absorption coefficient of 40,000 cm\(^{-1}\), which represents the highest value reported in prior research (Pettit & Ediger, 1996). It is clear that, regardless of the value used for the absorption coefficient, the blow-off model does not correctly predict ablation rates across the range of laser fluences.

![Figure 7-8](image)

Figure 7-8. Corneal tissue ablation rate as a function of laser fluence, compiled from several literature sources. Solid lines illustrate the results of using the static Beer-Lambert blow-off model for different values of the corneal tissue absorption coefficient.

In general, if the blow-off model were used with an absorption coefficient that produced very accurate predictions at low fluence, the model would increasingly overpredict the ablation rate as fluence increases. This is further evidence that there is a dynamic enhancement in tissue absorption during ablation, an effect that increases with increasing laser fluence and creates a deviation of the true ablation rate from the value predicted by a *static* blow-off model. In this section, a novel ablation model is developed to capture the physics of the ablation process as well as to predict ablation rates.
7.2.1 Formulation of the Ablation Model

The ablation model, which can be described as a Beer-Lambert dynamic absorption model, is similar to the traditional Beer-Lambert blow-off model in that it assumes that the excimer laser light is absorbed according the Beer-Lambert law as it penetrates into the corneal tissue. However, the absorption coefficient of corneal tissue is no longer static and is allowed to be a function of space and time. The dynamic absorption coefficient arises from the assumption that there are four species of interest in the corneal tissue during and following the ablation process. These four species are:

1. Collagen, which is divided into amino acid units (amino acid plus peptide bond)
2. Water
3. Transient absorbing species, formed by the interaction of collagen amino acid units with the excimer laser light
4. Stable and essentially non-absorbing product species, formed by the interaction of the transient absorbing species with surrounding water

This four-species model is considered a global model that represents the ablation mechanics as a whole rather than the fine details of the chemistry and individual reactions that might take place. This is a valid method for modeling complex processes that involve several reactions and intermediate species. Combustion processes are commonly modeled in this way, where the global representation is the reaction of a combustion fuel and an oxidizer (air or pure oxygen) to form carbon dioxide and water. In the dynamic ablation model, the transient absorbing species is assigned an absorption cross-section that is considered a representative average of all such species that may be present.

The potential complexities of geometry and time are simplified for the current model. First, the problem is considered one-dimensional in space such that all spatially dependent quantities are allowed to vary with depth (i.e., x-direction) into the tissue only. Second, the incident laser energy is assumed to be uniform in space, but varies with time.
using the probability distribution function \( p(t) \). The time variance is modeled by assuming a normal or Gaussian profile, which is considered quite reasonable for the excimer laser waveform. Figure 7-9 shows the initial setup of the model.

Figure 7-9. Geometry and setup of the dynamic corneal tissue ablation model. Species 1 is the amino acid unit in collagen and species 2 is water.

There are several variables and quantities that are incorporated into the dynamic ablation model that must be reviewed to understand the model itself. All of the important quantities, some of which have already been discussed in earlier chapters and some of which will be discussed throughout this chapter, are listed in Table 7-2.

Table 7-2. Variables and quantities used in the Beer-Lambert dynamic absorption ablation model.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I(x,t) )</td>
<td>Excimer laser photon flux (at location ( x ) and time ( t ))</td>
<td>photons/cm(^2)-s</td>
</tr>
<tr>
<td>( F )</td>
<td>Excimer laser fluence</td>
<td>J/cm(^2)</td>
</tr>
<tr>
<td>( p(t) )</td>
<td>Probability distribution function of laser energy</td>
<td>s(^{-1})</td>
</tr>
<tr>
<td>( S_i )</td>
<td>Species ( i ), used to represent a particular species in a reaction expression</td>
<td>N/A</td>
</tr>
<tr>
<td>( \rho_i(x,t) )</td>
<td>Number density of species ( i ) (at ( x ) and ( t ))</td>
<td>cm(^{-3})</td>
</tr>
<tr>
<td>( \rho_{hv}(x,t) )</td>
<td>Number density of 193-nm photons (at ( x ) and ( t ))</td>
<td>photons/cm(^3) (cm(^{-3}))</td>
</tr>
<tr>
<td>( \sigma_i )</td>
<td>Absorption cross-section of species ( i )</td>
<td>cm(^2)</td>
</tr>
<tr>
<td>( \alpha(x,t) )</td>
<td>Absorption coefficient of corneal tissue (at ( x ) and ( t ))</td>
<td>cm(^{-1})</td>
</tr>
<tr>
<td>( P )</td>
<td>Corneal tissue water fraction, by mass</td>
<td>Dimensionless (ranges from 0 to 1)</td>
</tr>
</tbody>
</table>
Keeping with the established species numbering convention, the absorption cross-section of the amino acid unit in collagen $\sigma_1$ is equal to $1.19 \times 10^{-17}$ cm$^2$ and the absorption cross-section of water $\sigma_2$ is equal to $4.5 \times 10^{-23}$ cm$^2$. These values were previously reported in Chapter 6. The average absorption cross-section of all transient absorbing species $\sigma_3$ is unknown, but is assumed to be larger than $\sigma_1$ ($\sigma_3 > \sigma_1$) to dynamically enhance the corneal tissue absorption coefficient. It is assumed that the average absorption cross-section of all essentially non-absorbing species $\sigma_4$ is much less than $\sigma_1$ ($\sigma_4 << \sigma_1$). The absorption coefficient of corneal tissue is the result of all four of these quantities since an absorption coefficient is by definition the product of the number density and the absorption cross-section of each species. Therefore, the corneal tissue absorption coefficient, in general, can be described by the following equation:

$$\alpha(x,t) = \rho_1(x,t)\sigma_1 + \rho_2(x,t)\sigma_2 + \rho_3(x,t)\sigma_3 + \rho_4(x,t)\sigma_4$$

(7-1)

However, scaling considerations can be used to argue that the second and fourth terms of Eq. 7-1 are negligible compared to the others. The absorption cross-section of water ($\sigma_2$) is six orders of magnitude lower than that of the amino acid unit in collagen ($\sigma_1$), while their respective number densities are likely to differ by no more than one or two orders of magnitude. The third term ($\rho_3\sigma_3$) is considered important because, although the concentration of species 3 is expected to be small, the absorption coefficient is assumed sufficiently large to make this term relevant and, in fact, solely responsible for the transient enhancement in tissue absorption. Both the number density and absorption cross-section of species 4 are expected to be much less than those for the amino acid unit in collagen and for water, making the product very small and therefore negligible.
The global mechanism of the dynamic ablation model is based on two primary reactions. The first is the interaction of the excimer laser light with the amino acid unit in collagen (species 1), which is labeled Reaction I:

\[
(RI) \quad S_1 + n(h\nu)_{93} \xrightarrow{k_i} S_3 \quad (7-2)
\]

In this relation, the “\(n(h\nu)_{93}\)” term represents the absorption of \(n\) excimer laser photons by the amino acid unit. It should be noted that, although Reaction I does not explicitly involve water, the implicit assumption of the proposed dynamic absorption model is the interaction of transient species with water, including the possibility of multiple reaction steps and pathways. Without water present in the initial tissue matrix, the situation would simplify to the case of dry collagen, for which the static blow-off model has been shown to be a valid predictor of ablation. Water must be present for Reaction I of the global mechanism to proceed. The rate expression for this reaction can be written as follows:

\[
\frac{\partial \rho_1(x,t)}{\partial t} = -\rho_1(x,t)\rho_{n_i}^n k_i \quad (7-3)
\]

The second reaction is the interaction between the transient absorbing species (species 3) and water to produce a stable product (species 4), and it is labeled Reaction II:

\[
(RII) \quad S_3 + S_2 \xrightarrow{k_{II}} S_4 \quad (7-4)
\]

The rate expression for this reaction can be written as follows:

\[
\frac{\partial \rho_2(x,t)}{\partial t} = -\frac{\partial \rho_3(x,t)}{\partial t} = -\rho_2(x,t)\rho_3(x,t)k_{II} \quad (7-5)
\]

Furthermore, since species 3 appears in both Reactions I and II (Eqs. 7-2 and 7-4), its time rate of change can be expressed as follows:

\[
\frac{\partial \rho_3(x,t)}{\partial t} = \rho_1(x,t)\rho_{n_i}^n k_i - \rho_2(x,t)\rho_3(x,t)k_{II} \quad (7-6)
\]
To complete the formulation, the Beer-Lambert law is used to describe the relationship between the photon density and the depth into the tissue. The differential form of the Beer-Lambert law can be expressed as follows:

\[ d\phi = -(\alpha \phi) \, dx \quad (7-7) \]

However, the photon flux \( I(x,t) \) is directly related to the power \( \phi \) using quantities that are constant with respect to \( x \). Furthermore, the photon density \( \rho_{hv} \) is related to the flux using quantities that are also constant with respect to \( x \). Therefore, the Beer-Lambert law can be rewritten as follows:

\[ \frac{\partial \rho_{hv}}{\partial x} = -\alpha \rho_{hv} \quad (7-8) \]

To obtain the final, useful form of the Beer-Lambert law, it is noted that the absorption coefficient \( \alpha \) is for corneal tissue, which was already described in Eq. 7-1. The final form of the Beer-Lambert law for the purposes of the dynamic absorption model is therefore expressed, in general, as follows:

\[ \frac{\partial \rho_{hv}(x,t)}{\partial x} = -[\rho_1(x,t)\sigma_1 + \rho_2(x,t)\sigma_2 + \rho_3(x,t)\sigma_3 + \rho_4(x,t)\sigma_4] \rho_{hv}(x,t) \quad (7-9) \]

### 7.2.2 Initial and Boundary Conditions

From the above formulation, it is clear that there are five quantities of interest that vary with both space and time—the number densities of the four species in the cornea and the photon number density. To proceed with the model, it is necessary to determine the initial and/or boundary conditions for these quantities. The simplest conditions are imposed on species 3 and 4, which are formed during or after ablation. By definition, since they do not exist prior to the onset of ablation, these species have initial number densities equal to zero. These conditions are represented mathematically as follows:
\[ \rho_3(x, t = 0) = \rho_{30} = 0 \]  \hfill (7-10)

\[ \rho_4(x, t = 0) = \rho_{40} = 0 \]  \hfill (7-11)

For the amino acid unit in collagen (species 1), the initial number density depends on the mass fraction of water in corneal tissue. Typically, corneal tissue is assumed to be 80% water and 20% collagen by mass. However, in order to investigate the effects of corneal hydration on the ablation rate, the water mass fraction was left as a variable \( P \). Referring to Eq. 2-8, it was determined that there are \( 6.77 \times 10^{21} \) peptide bonds per gram of collagen, which is also equal to the number of amino acid units per gram of collagen. Furthermore, corneal tissue is assumed to have a mass density of 1 g/cm\(^3\) since it consists primarily of water. Therefore, the initial number density of the amino acid unit can be determined as follows:

\[ \rho_1(x, t = 0) = \rho_{10} = (1 - P) \left( \frac{1}{cm^3} \right) \left( 6.77 \times 10^{21} \text{ g}^{-1} \right) \]  \hfill (7-12)

The initial number density of water molecules also depends on the water mass fraction. Using the Avogadro constant and the molecular weight of water, it can be determined that there are \( 3.34 \times 10^{22} \) molecules per gram of water. Again assuming a corneal tissue mass density of 1 g/cm\(^3\), the initial number density of water molecules can be determined as follows:

\[ \rho_2(x, t = 0) = \rho_{20} = P \left( \frac{1}{cm^3} \right) \left( 3.34 \times 10^{22} \text{ g}^{-1} \right) \]  \hfill (7-13)

Only the imposed condition for the photon number density remains. It was stated in the formulation of the model that the incident laser energy was assumed to be spatially uniform but time-dependent. The time dependence is described by the assumption of a
normal or Gaussian distribution. The general form of the probability density function for a normal distribution is as follows:

\[
p(t) = \frac{1}{s_{\text{dev}} \sqrt{2\pi}} \exp \left[ -\frac{(t - t_{\text{avg}})^2}{2s_{\text{dev}}^2} \right]
\] (7-14)

In this distribution function, \(t_{\text{avg}}\) is the mean and \(s_{\text{dev}}\) is the standard deviation. These quantities are determined by known parameters of the laser pulse and its time profile.

First, it must be recognized that a normal distribution exists over an infinite domain (both positive and negative) while the true laser pulse has a finite existence. However, nearly 100% of the information for a normal distribution is contained within \(t_{\text{avg}} \pm 3s_{\text{dev}}\).

Therefore, the following condition was imposed:

\[
t_{\text{avg}} - 3s_{\text{dev}} = 0
\] (7-15)

Furthermore, the excimer laser pulse has a full-width of approximately 25 nanoseconds (ns) and a full-width half-maximum value (FWHM) of approximately 10 ns.

Conveniently, assigning values of 12.6 ns and 4.2 ns for \(t_{\text{avg}}\) and \(s_{\text{dev}}\), respectively, gives a FWHM of 9.89 ns and satisfies Eq. 7-15 as well as the condition that the laser pulse ends at approximately 25 ns.

The photon flux at the surface of the cornea is directly related to the laser fluence. Assuming the normal distribution already discussed, a laser fluence \(F\), and a photon energy equal to the product of Planck’s constant \((h=6.626 \times 10^{-34} \text{ J-s})\) and the frequency \((\nu)\), the relationship between photon flux and laser fluence is as follows:

\[
I(x = 0, t) = F \left( \frac{1}{h \nu} \right) p(t)
\] (7-16)
Photon number density is related to photon flux using the speed of light in corneal tissue \( (c_{\text{cornea}}) \), since the photons propagate through the corneal tissue. Therefore, the photon number density at the corneal surface can be expressed as follows:

\[
\rho_{hv}(x = 0, t) = \frac{F(x = 0, t)}{c_{\text{cornea}}} = \left( \frac{n_{\text{cornea}}}{c_{\text{vacuum}}} \right) \left( \frac{1}{h \nu} \right) \rho(t)
\]  

(7-17)

The photon number density at the corneal surface, then, can be expressed in its entirety using the following relation:

\[
\rho_{hv}(x = 0, t) = \left( \frac{F}{h \nu} \right) \left( \frac{n_{\text{cornea}}}{c_{\text{vacuum}}} \right) \left( \frac{1}{(4.2 \, \text{ns})\sqrt{2\pi}} \right) \exp \left[ -\frac{(t - 12.6 \, \text{ns})^2}{2 (4.2 \, \text{ns})^2} \right]
\]  

(7-18)

This condition can also be shown graphically by plotting \( \rho_{hv}(x=0,t) \) as a function of time. To do this, values for \( F \) and \( n_{\text{cornea}} \) must be assumed. The known values for \( h \) and \( c_{\text{vacuum}} \) are \( 6.626 \times 10^{-34} \) J-s and \( 2.998 \times 10^{10} \) cm/s, and the frequency is calculated from the following:

\[
\nu = \frac{c}{\lambda} = \frac{2.998 \times 10^8 \, \text{m}}{193 \times 10^{-9} \, \text{m}} \approx 1.55 \times 10^{15} \, \text{s}^{-1}
\]  

(7-19)

A plot of the photon density time distribution at the corneal surface is shown in Figure 7-10. A clinically relevant value of 350 mJ/cm\(^2\) was chosen for the fluence \( F \), and the refractive index of corneal tissue \( n_{\text{cornea}} \) was assumed to be 1.4.
Figure 7-10. Time profile of photon density at the corneal surface (x=0). The mean time ($t_{avg}$) of 12.6 ns and the full-width half-maximum (FWHM) value of 9.89 ns are indicated.

### 7.2.3 Estimating the Reaction Rate Constants

The reaction rate constants, $k_1$ and $k_2$, are critical parameters that must first be estimated in order to implement this ablation model. First-order estimates of these constants can be obtained using the initial values of the species number densities $\rho_1$, $\rho_2$, $\rho_3$, and $\rho_4$ under typical conditions and a relevant value of the photon density $\rho_{hv}$.

Assuming a typical corneal constituency of 20% collagen and 80% water and using Eqs. 7-12 and 7-13, the species number densities $\rho_1$ and $\rho_2$ can be estimated as $1.4 \times 10^{21}$ cm$^{-3}$ and $2.7 \times 10^{22}$ cm$^{-3}$, respectively. As discussed in Section 7.2.2, the initial values of $\rho_3$ and $\rho_4$ are both zero. Assuming a laser fluence of 350 mJ/cm$^2$ and a corneal tissue refractive index of 1.4, and using the peak value of the laser pulse distribution function (at $t=12.6$ ns), Eq. 7-18 can be used to estimate a photon density $\rho_{hv}$ of $1.5 \times 10^6$ cm$^{-3}$.
Note that $\rho_{hv}$ is orders of magnitude lower than $\rho_1$ and $\rho_2$, according to these estimates, which makes $\rho_{hv}$ negligible when estimating the reaction time constants.

Recalling Eq. 7-5, it is clear that the rate of change in the number density of species 2 (water) is dependent on $\rho_2$ and $\rho_3$. However, $\rho_3$ is initially zero and will likely remain much smaller than $\rho_2$ throughout the ablation process. Therefore, a first-order estimate of the time constant for Reaction II (Eq. 7-4) is:

$$\tau_{II} = \frac{1}{\rho_2 k_{II}}$$

(7-20)

Using similar logic for Reaction I (Eq. 7-2), where $\rho_1$ is much larger than $\rho_{hv}$, and assuming that the order of the reaction is unity (i.e., $n=1$), a first-order estimate of the time constant for Reaction I is:

$$\tau_I = \frac{1}{\rho_1 k_I}$$

(7-21)

Assuming that $n$ is unity is equivalent to assuming that the photochemical reaction is a single-photon absorption process. This makes subsequent calculations and estimates much more convenient, and is considered a valid assumption, given the high photon energy ($\sim 6.3$ eV) of 193-nm radiation. All that remains to determine first-order estimates of $k_I$ and $k_{II}$, then, is to estimate the relative time scales of Reactions I and II. Reaction I is considered a prompt process that certainly occurs during the course of the laser pulse, and likely begins near the start of the pulse, if the model is to in fact predict transient changes in reflectivity as observed. Since the FWHM of the laser pulse is approximately 10 ns, $\tau_I$ was estimated to be on the order of 1 ns. Reaction II, on the other hand, is considered a slow process that very likely lags far behind the laser pulse. Again using the FWHM of the laser pulse as a basis, $\tau_{II}$ was estimated to be on the order of 1000 ns (i.e.,
These values, which themselves are \textit{ab initio} estimates, determine the first-order estimates of the reaction rate constants as shown below:

\begin{align*}
{k}_I &= \frac{1}{\rho_1 \tau_I} = \frac{1}{(1.4 \times 10^{21} \text{ cm}^{-3})(1 \times 10^{-9} \text{ s})} \approx 7.1 \times 10^{-13} \frac{\text{ cm}^3}{\text{s}} \\
{k}_{II} &= \frac{1}{\rho_2 \tau_{II}} = \frac{1}{(2.7 \times 10^{22} \text{ cm}^{-3})(1 \times 10^{-6} \text{ s})} \approx 3.7 \times 10^{-17} \frac{\text{ cm}^3}{\text{s}}
\end{align*}

\subsection*{7.2.4 Summary of Relevant Equations and Conditions}

There are five partial differential equations that form the basis for the current dynamic Beer-Lambert absorption model of corneal tissue ablation. These equations, all of which have appeared in some form previously in this chapter, are summarized below in their general forms:

\begin{align*}
(1) \quad & \frac{\partial \rho_1(x,t)}{\partial t} = -\rho_1(x,t)\rho_{hv}^*(x,t)k_I \\
(2) \quad & \frac{\partial \rho_2(x,t)}{\partial t} = -\rho_2(x,t)\rho_3(x,t)k_{II} \\
(3) \quad & \frac{\partial \rho_3(x,t)}{\partial t} = \rho_1(x,t)\rho_{hv}^*(x,t)k_I - \rho_2(x,t)\rho_3(x,t)k_{II} \\
(4) \quad & \frac{\partial \rho_4(x,t)}{\partial t} = -\frac{\partial \rho_2(x,t)}{\partial t} = \rho_2(x,t)\rho_3(x,t)k_{II} \\
(5) \quad & \frac{\partial \rho_{hv}(x,t)}{\partial x} = -[\rho_1(x,t)\sigma_1 + \rho_2(x,t)\sigma_2 + \rho_3(x,t)\sigma_3 + \rho_4(x,t)\sigma_4] \rho_{hv}(x,t)
\end{align*}

The reaction rate constants are critical to the solution of the above equations. As shown in Section 7.2.3, first-order estimates are $7.1 \times 10^{-13} \text{ cm}^3/\text{s}$ and $3.7 \times 10^{-17} \text{ cm}^3/\text{s}$ for
\( k_1 \) and \( k_2 \), respectively. The relevant initial and boundary conditions required for solution to the above system of partial differential equations are summarized below:

\[
(1) \quad \rho_1(x, t = 0) = \rho_{10} = \left(1 - P\right) \left(6.77 \times 10^{21} \ \text{cm}^{-3}\right)
\]

\[
(2) \quad \rho_2(x, t = 0) = \rho_{20} = P \left(3.34 \times 10^{22} \ \text{cm}^{-3}\right)
\]

\[
(3) \quad \rho_3(x, t = 0) = \rho_{30} = 0
\]

\[
(4) \quad \rho_4(x, t = 0) = \rho_{40} = 0
\]

\[
(5) \quad \rho_{hv}(x = 0, t) = \left(\frac{F}{h \nu}\right) n_{\text{cornea}} \frac{1}{s_{\text{dev}} \sqrt{2\pi}} \exp\left[-\frac{(t - t_{\text{avg}})^2}{2 s_{\text{dev}}^2}\right]
\]

For the boundary condition on the photon number density (Eq. 7-33), \( h, \nu, n_{\text{cornea}}, \) and \( c_{\text{vacuum}} \) were defined already. The values of \( t_{\text{avg}} \) and \( s_{\text{dev}} \) are 12.6 ns and 4.2 ns, respectively, to create an excimer laser time profile with the proper characteristics. The values of the laser fluence (\( F \)) and the corneal tissue water mass fraction (\( P \)) are inputs to the system that must be specified, and are left as variables to allow investigation of the effects of these parameters on the ablation process.

### 7.2.5 Theoretical Framework and Physics of the Model

The numerical solution of the dynamic absorption model of corneal tissue ablation presented in this chapter was not included within the scope of current work. However, an examination of the validity of the model is useful to determine if it makes physical sense. The first, and perhaps most important, aspect to examine is the relationship between laser fluence and ablation rate. As reported in Chapter 5, experimental evidence supports a positive relationship between laser fluence and ablation rate (i.e., rate increases with increasing fluence). However, as discussed at the beginning of Section 7.2, experimental evidence has shown that although the corneal tissue ablation rate increases with
increasing laser fluence, the degree of increase in the rate becomes incrementally smaller. In other words, the rate curve flattens out and the ablation rate, in effect, tends to somewhat “level off” at high laser fluence, a phenomenon that was shown in Figure 7-8.

In the current ablation model, the laser fluence appears in the boundary condition for the photon number density (Eq. 7-33). As laser fluence increases, the photon number density at the surface of the cornea also increases for all time, which logically leads to deeper laser penetration into the tissue. Deeper laser penetration is of course indicative of a larger ablation rate, which is expected. More importantly, however, the increase in photon number density leads to more production of species 3 (according to Reaction I), which in turn leads to a greater consumption of photons according to Eq. 7-9. Essentially, an increased production of species 3 is analogous to a transiently enhanced tissue absorption coefficient, which partially counterbalances the increase in the photon density and leads to an ablation rate that is, while still larger, not as large as would be predicted if a static blow-off model were used. Therefore, the model does indeed appear to capture the physics of the relationship between laser fluence and tissue ablation rate, including both an increase in rate and a downward deviation (i.e., dynamic absorption enhancement) from static Beer-Lambert behavior as laser fluence increases.

The second aspect is the effect of corneal tissue hydration on the ablation rate. Prior research has supported both a positive and a negative relationship between hydration and ablation rate, while this work has produced experimental evidence of a positive relationship between the two (i.e., rate increases with increasing hydration). Based on the summary of the current work and previous studies, the overall conclusion is that the ablation rate is expected to increase with increasing hydration. In the current
ablation model, tissue hydration is defined by the initial conditions for $\rho_1$ and $\rho_2$ (Eqs. 7-29 and 7-30). As tissue hydration increases, the water fraction ($P$) increases, which in turn corresponds to a decrease in $\rho_{10}$ and an increase in $\rho_{20}$. A decrease in $\rho_{10}$ means that there are fewer 193-nm chromophores, and a subsequent decrease in the production of species 3, the transient absorbing species. At the same time, an increase in $\rho_{20}$ means greater consumption of species 3 (based on Reaction II). In concert, these two processes result in a decrease in $\rho_3$, which in turn allows greater penetration of the laser into the tissue. This can be seen in Eq. 7-28, where a decrease in $\rho_3$ dictates a decrease in the rate of consumption of photons, meaning that photons penetrate deeper.

In terms of the physics, the overall effect of increasing hydration is a decrease in the transient enhancement of the absorption coefficient of corneal tissue due to a decrease in the availability of the highly absorbing transient species (species 3). A decrease in the transient enhancement of absorption leads to an increase in laser penetration depth and therefore a higher ablation rate. Therefore, ablation rate increases with increasing corneal tissue hydration according to the current dynamic ablation model. This is consistent with experimental evidence presented both in this work and in prior research.

7.2.6 Summary of Modeling Efforts

A global model of corneal tissue ablation has been presented. It is based on the Beer-Lambert law, but it is a dynamic model in that it allows the absorption coefficient of corneal tissue to be a function of both space and time. This absorption coefficient is determined primarily by two species—the amino acid unit in collagen and a representative, average transient species characterized by enhanced absorption of 193-nm excimer laser light. The model also includes water and a fourth species, which is
considered a stable product and functions to destroy the transient absorbing species over a delayed timeframe. This is consistent with previous research, which shows that changes in corneal surface reflectivity decay with time after the excimer laser pulse.

Mathematically, the current ablation model is a system of five partial differential equations that must be solved simultaneously using four initial conditions and a boundary condition. There appears to be no analytical solution to this system of equations, which means that numerical methods must be used to obtain an approximate solution. The solution of this system of equations is not included within the scope of this dissertation, but it is believed that this model is reasonable and captures the physics of the ablation process. Future work should include solution of the system of equations to fully realize the model.
CHAPTER 8
SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS FOR FUTURE WORK

Despite the wide popularity and generally satisfactory results of clinical refractive procedures such as PRK and LASIK, the process of excimer laser corneal tissue ablation is not well understood. Furthermore, current clinical laser systems include no method for real-time feedback of the true ablation rate during the procedure; hence there is no comparison to the empirical average ablation rate that is used to determine the treatment algorithm. The body of research encompassed in this dissertation was devoted to two primary goals—the basic scientific goal of understanding the mechanisms of corneal tissue ablation and the interactions between the excimer laser light and the tissue, and the more practical goal of developing diagnostic tools to provide ablation rate feedback during clinical refractive procedures. Ultimately, the goal was to incorporate all of the knowledge gained in this research with existing knowledge and theory to develop a comprehensive model of the corneal tissue ablation process that can be used for both illustrative and predictive purposes.

8.1 Summary and Conclusions

Experimentation involved the use of two ArF excimer lasers, primarily used separately but sometimes in conjunction, and a vast array of optics and detectors in several different excitation and detection configurations. The various achievements and important conclusions of this research are highlighted below:
1. Raman spectroscopy, using a confocal excitation-detection system, was demonstrated as an effective method for measuring corneal hydration. Using the confocal Raman technique, it was determined that corneal tissue hydration can decrease significantly over relatively short, clinically relevant time scales. The degree of dehydration was found to be much greater when the tissue was exposed to a moderate cross-flow of nitrogen gas than when the tissue was exposed only to nominally quiescent surrounding air, a finding that is particularly relevant to LASIK because the procedure involves the use of a suction tube that induces a flow field above the surface of the eye. Furthermore, a great deal of insight was gained into the processes of diffusion within the corneal tissue and mass transfer to the surroundings, both of which contribute to dehydration of corneal tissue.

2. A novel technique to measure the corneal tissue ablation rate, using wax molds of ablation craters and white-light interferometry, was developed and successfully implemented. PMMA, a common polymer, was used to verify that the depth and ablation rate determined from a wax impression matches those determined directly from the original, ablated surface. Because corneal tissue is transparent, deformable, and susceptible to changes in shape due to dehydration, it was impossible to examine tissue ablation craters directly with the interferometer. This limitation was the impetus for this new technique.

3. The cross-correlation of the incident laser pulse waveform (time-intensity profile) and the promptly reflected pulse waveform was demonstrated, and was determined to be useful for quantifying changes in the reflected waveform that arise during ablation. Previous research has shown, and the current research indeed verified, that the reflected waveform shape is truncated on the trailing edge during ablation as compared to the shape when the laser fluence is below the ablation threshold. Two quantities were extracted from the cross-correlation function—the initial slope, or the slope of the initial, typically increasing portion of the function, and the decay slope, or the slope of the decaying, approximately linear portion of the function. The decay slope was determined to be more sensitive than the initial slope to changes in the reflected waveform shape.

4. The decay slope of the cross-correlation function was shown to positively correlate with the corneal tissue ablation rate (as measured with the novel wax impression-interferometry technique). That is, the decay slope increases with increasing ablation rate. Furthermore, this relationship was shown to be linear, albeit with some amount of scatter in the data. This is an encouraging finding, as it suggests that the decay slope, which can be obtained passively with little modification to current clinical laser systems, can be used as a tool for measuring the ablation rate.

5. A positive correlation between the corneal tissue ablation rate and corneal tissue hydration was demonstrated, despite a limited amount of data. Previous research is indecisive and self-contradictory, meaning that these quantities have been shown to be both positively and negatively correlated, depending on the source. Overall, the current work suggests that the ablation rate increases monotonically with corneal hydration.
6. It was shown that the absorption coefficient of water during laser irradiation is nearly constant and does not deviate from the small-signal value (0.15 cm\(^{-1}\)) over the range of fluences studied. This finding suggests that the interaction of excimer laser light with water alone does not contribute to any dynamic photochemistry.

7. For the first time, the assumption that peptide bonds in collagen are the primary chromophores for 193-nm excimer laser light was verified with direct experimental evidence. From transmission measurements, it was determined that the peptide bond is responsible for 96% of the absorption of excimer laser light in collagen, while the amino acids themselves are responsible for the other 4%. The absorption cross-section of the amino acid unit in collagen (amino acid plus peptide bond) was calculated as 1.19 \(\times 10^{17}\) cm\(^2\), which results in an equivalent corneal tissue absorption coefficient of 16,000 cm\(^{-1}\). Previous research has shown that the Beer-Lambert blow-off model requires a static tissue absorption coefficient of 20,000-30,000 cm\(^{-1}\) to accurately predict tissue ablation rates, which is consistent with a reasonable 25-75% dynamic enhancement of the current value (16,000 cm\(^{-1}\)).

8. Ablation experiments using thin, dry collagen films showed that the ablation rate of dry collagen increases with increasing laser fluence. The Beer-Lambert blow-off model was used to predict the ablation rate of dry collagen using the experimentally measured absorption cross-section and a known peptide bond density. Predicted ablation rates matched the experimental ablation rates very well within the experimental uncertainty, suggesting that the blow-off model is an adequate method for modeling the ablation rate of dry collagen (i.e., no water present).

9. Observations of emission from corneal tissue during and after the excimer laser pulse provided experimental evidence that the formation of a laser-induced plasma is unlikely, even for laser fluences far exceeding clinical relevance. Furthermore, the spectral data suggested that the observed atomic emission was very likely fluorescence arising from photofragmentation or some other non-thermal process. The body of prior ablation research includes a limited number of spectral investigations reporting little useful information. Generally, essential signal timing parameters are omitted and/or the spectra were obtained only for extremely high laser fluence because of a lack of appreciable signal at clinically relevant fluences. Spectra obtained during the course of this doctoral research were obtained with high spectral resolution and were spectrally normalized using calibrated emission sources. This work is believed to be the first to contain relevant and useful fluorescence spectra obtained at clinically relevant laser fluences, with appropriate signal gate timing, and with relatively high spectral resolution.

10. A global model of corneal tissue ablation was formulated. Based on the Beer-Lambert law, this model included a dynamic tissue absorption coefficient in an attempt to capture the dynamic nature of the physics of laser tissue ablation. A system of five partial differential equations was developed to model the ablation process and appropriate initial and boundary conditions were defined. The model was shown to explain the effects of laser fluence and corneal tissue hydration in a manner that is consistent with the experimental evidence presented in this work.
8.2 Recommendations for Future Work

A great deal of work has been completed with mostly positive and useful outcomes, as described in Section 8.1. One of the primary goals of this research was to achieve a better understanding of the excimer laser corneal tissue ablation process, a goal that was achieved. Nevertheless, a complete and comprehensive understanding of the ablation process has not yet been attained and requires future work. With that in mind, recommendations for future corneal tissue ablation research are proposed below:

1. The initial and decay slopes of the cross-correlation function have been identified as quantities that effectively quantify the degree of truncation of the reflected waveform, and the decay slope has been shown to have greater sensitivity. However, other quantities (e.g., initial value, peak value, 50% decay point) can be extracted from the cross-correlation function, and these should be explored to determine if any of them are more sensitive than the decay slope.

2. Assessment of corneal hydration using a confocal Raman technique was found to be effective. Due to the nature of the instrumentation, however, the reported hydration measurements were essentially volume-integrated and representative of the bulk corneal tissue. Only a small portion of the tissue was actually exposed to the outside environment, meaning that only the portions of the tissue nearest the exposed surface were expected to significantly dehydrate. Therefore, future experiments should be devoted to developing or implementing a more surface-weighted Raman measurement technique to enhance the sensitivity of the hydration measurements. This may be accomplished by pushing the confocal limits of the Raman system.

3. The confocal Raman technique used in this work could not be used to obtain in situ hydration measurements during excimer laser ablation. Corneal hydration was inferred through interpolation between Raman measurements taken immediately prior to and following ablation. It would be beneficial to develop an in situ Raman measurement technique for corneal hydration measurements during ablation. This would likely require another laser, in addition to the ablating excimer laser, that would have to be accurately timed and carefully aligned with respect to the excimer laser to obtain relevant hydration measurements.

4. If a suitable in situ Raman technique is successfully developed to measure corneal hydration during ablation, future work should certainly include a statistically significant set of measurements of corneal tissue hydration for constant excimer laser energy. Such measurements would elucidate the effect of corneal hydration on the tissue ablation rate with greater confidence than currently exists.
5. A definite, positive correlation between the decay slope of the cross-correlation function and the corneal tissue ablation rate was demonstrated. However, future work must be devoted to determining exactly how to integrate the cross-correlation method into clinical laser systems. The cross-correlation function is formulated from data that is taken passively, using the already existing excimer laser and requiring minimal optics and detectors. However, the exact collection geometry that minimizes the degree of modification to clinical laser systems and maximizes the detected signals remains to be determined.

6. Ablation-induced emission experiments showed that excimer laser ablation of corneal tissue is not likely to involve any type of laser-induced plasma and is much more likely to involve non-thermal processes such as prompt fluorescence and photofragmentation. Additional laser-induced fluorescence (LIF) experiments are recommended to attempt to identify and possibly track any transient species that might form during corneal tissue ablation. Based on the availability of the constituent atoms and knowledge of some of its properties, formaldehyde (CH₂O) is considered a possible candidate for a transient species that could strongly absorb excimer laser light and contribute to a dynamically enhanced corneal tissue absorption coefficient. Such experiments will require another laser, in addition to the ablating excimer laser, that must be tunable over a wide range of wavelengths to probe various species. This can be realized with the use of an optical parametric oscillator (OPO) pumped by a high-power source (e.g., a pulsed Nd:YAG laser).

7. A global model of corneal tissue ablation, including a dynamically enhanced tissue absorption coefficient, was presented and was demonstrated to be consistent with current experimental evidence in terms of the effects of laser fluence and corneal hydration on the ablation rate. However, the model is formulated using a system of five partial differential equations that remains to be solved. An analytical solution appears unlikely, leaving numerical methods as the best candidate for achieving a solution. Future work should include efforts to solve the system of equations, most likely using a finite-difference numerical technique applied in a computer program such as Matlab. If possible, a program should be written to allow rapid solution of the system when given appropriate input values such as the laser fluence and the tissue water fraction. This would allow the programmer to obtain the corneal tissue ablation rate as a function of laser fluence and would facilitate a comparison of these results to the multitude of experimental data (rate vs. fluence and rate vs. hydration) available in the literature, as well as that presented in this work.
APPENDIX A
REFRACTIVE INDEX OF WATER

The refractive index of water was calculated based on the formulation presented in a publication by Harvey, Gallagher & Leveit Sengers (1998). The following is a list of assumptions that were made for this formulation:

1. \( T = 25^\circ C = 298 \text{ K} \) (true temperature)
2. \( \rho = 996.5 \text{ kg/m}^3 \) (mass density)
3. \( \lambda = 193 \text{ nm} = 0.193 \text{ \mu m} \) (wavelength of light)
4. \( T^* = 273.15 \text{ K} \) (reference temperature, given in publication)
5. \( \rho^* = 1000 \text{ kg/m}^3 \) (reference density, given)
6. \( \lambda^* = 0.589 \text{ \mu m} \) (reference wavelength, given)

Using these values, it was possible to calculate dimensionless quantities. These quantities, according to formulas given by Harvey et al. (1998), were calculated as:

1. \( \bar{T} = T / T^* \approx 1.091 \)
2. \( \bar{\rho} = \rho / \rho^* \approx 0.9965 \)
3. \( \bar{\lambda} = \lambda / \lambda^* \approx 0.3277 \)

It was then possible to use the equation presented in Harvey et al. (1998) to solve for the refractive index of water. This equation is shown below:

\[
\frac{n^2 - 1}{n^2 + 2} \left( \frac{1}{\rho} \right) = a_0 + a_1 \bar{\rho} + a_2 \bar{T} + a_3 \bar{\lambda}^2 \bar{T} + \frac{a_4}{b} + \frac{a_5}{c} + a_7 \rho^2
\]  

(A-1)

The quantities \( b \) and \( c \) are calculated according to the following:

\[
b = \bar{\lambda}^2 - \lambda_{UV}^2
\]  

(A-2)

\[
c = \bar{\lambda}^2 - \lambda_{IR}^2
\]  

(A-3)

The coefficients required to complete the calculation \((a_0, a_1, a_2, a_3, a_4, a_5, a_6, a_7, \lambda_{UV} \text{ and } \lambda_{IR})\) can all be found in Harvey et al. (1998).
Determining the right-hand side of Eq. A-1 was a simple matter, and the result was the following:

\[ \frac{n^2 - 1}{n^2 + 2} = 0.2614881 \]  \hspace{1cm} \text{(A-4)}

A simple rearrangement of Eq. A-4 led to the final result:

\[ n_{water} \approx 1.436 \]  \hspace{1cm} \text{(A-5)}
APPENDIX B
TRANSMISSION CORRECTION USING FRESNEL THEORY

It is necessary, when determining the true transmission through a given material, to account for surface reflections due to mismatches in refractive index, known as Fresnel losses. Assuming the medium into which the light is entering has real refractive index $n_2$, the medium from which the light is coming has real refractive index $n_1$, and the light crosses between the media at normal incidence, then Fresnel theory states that the reflectivity is as follows:

$$\rho = \left(\frac{n_2 - n_1}{n_2 + n_1}\right)^2 \tag{B-1}$$

At the interface between two media, light can either be reflected or transmitted. Therefore, reflectivity and transmissivity must sum to unity. Transmissivity can then be expressed as follows:

$$\tau = 1 - \rho \tag{B-2}$$

Eqs. B-1 and B-2 apply when there is one surface or interface. When there are two or more interfaces, it is necessary to multiply the individual reflectivities together to get the overall reflectivity. Likewise, one can multiply the individual transmissivity values together to get the overall transmissivity corrected for Fresnel losses. For example, if light travels through a medium ($n_1$), then enters a new medium ($n_2$) and travels through it and back out into the original medium, the overall transmissivity would be as follows:
\[
\tau = \left[ 1 - \left( \frac{n_2 - n_1}{n_2 + n_1} \right)^2 \right]^2
\]  

(B-3)

Therefore, it was possible to correct the transmission of water, both in a quartz cuvette and as a film on a quartz flat, using these relationships. It was assumed that air has refractive index \( n=1.0 \), quartz has a refractive index \( n=1.56 \), and water has a refractive index \( n=1.436 \). Details concerning the determination of the refractive index of water can be found in Appendix A.

When passing through an empty cuvette, laser light encounters four air-quartz interfaces where Fresnel losses occur. This configuration is illustrated in Figure B-1.

![Figure B-1](image)

Figure B-1. Top view of empty cuvette showing the transmission of excimer laser light (large arrow) and the Fresnel reflection losses at the four quartz-air interfaces (small arrows).

The transmissivity in this situation is calculated as follows:

\[
\tau = \left[ 1 - \left( \frac{1.56 - 1.0}{1.56 + 1.0} \right)^2 \right]^4 \approx 0.8219
\]  

(B-4)

When passing through a cuvette containing water, laser light encounters two air-quartz interfaces and two quartz-water interfaces. The configuration is the same as that shown in Figure B-1, but with the interior of the cuvette filled with water and the two innermost Fresnel reflections resulting from boundaries between quartz and water. The transmissivity in this situation is calculated as follows:
When passing through a clean quartz flat, laser light encounters two quartz-air interfaces, as shown in Figure B-2.

![Figure B-2](image-url)

This configuration leads to a transmissivity of:

\[
\tau = \left[ 1 - \left( \frac{1.56 - 1.0}{1.56 + 1.0} \right)^2 \right]^2 \left[ 1 - \left( \frac{1.56 - 1.436}{1.56 + 1.436} \right)^2 \right] \approx 0.9035 \tag{B-5}
\]

Finally, when passing through a water film on top of a quartz flat, laser light encounters one air-water interface, one water-quartz interface, and one quartz-air interface, as shown in Figure B-3.

![Figure B-3](image-url)

The transmissivity in this situation is calculated as follows:

\[
\tau = \left[ 1 - \left( \frac{1.436 - 1.0}{1.436 + 1.0} \right)^2 \right]^2 \left[ 1 - \left( \frac{1.56 - 1.436}{1.56 + 1.436} \right)^2 \right] \left[ 1 - \left( \frac{1.56 - 1.0}{1.56 + 1.0} \right)^2 \right] \approx 0.9201 \tag{B-7}
\]
To obtain the corrected transmission of laser light passing through a water-filled cuvette, the experimentally measured transmission should be corrected using Eqs. B-4 and B-5. The result is as follows:

$$\tau_{\text{corrected, cuvette}} = \frac{\tau_{\exp}}{0.9035/0.8219} = \tau_{\exp} \left(\frac{0.8219}{0.9035}\right)$$ \hspace{5cm} (B-8)

Similarly, to obtain the corrected transmission of laser light passing through a thin water film on a quartz flat, the experimentally measured transmission should be corrected using Eqs. B-6 and B-7. The result is as follows:

$$\tau_{\text{corrected, film on flat}} = \frac{\tau_{\exp}}{0.9201/0.9066} = \tau_{\exp} \left(\frac{0.9066}{0.9201}\right)$$ \hspace{5cm} (B-9)
REFERENCES


BIOGRAPHICAL SKETCH

Brian T. Fisher, the second of two children, was born on November 3, 1978, in Springfield, Massachusetts, to parents Lee and Susan Fisher. His family moved to Sarasota, Florida, in 1980, where Brian remained until he graduated from high school in 1996. Brian graduated with highest honors from the University of Florida in August 2000, obtaining a Bachelor of Science in Mechanical Engineering with a minor in business administration. He immediately began graduate school in pursuit of a Ph.D. in mechanical engineering, with his doctoral research funded by the Stephen C. O’Connell Presidential Fellowship from the University of Florida. In January 2005, Brian will begin postdoctoral research work at the Naval Research Laboratory in Washington, D.C., funded by a two-year National Research Council Research Associateship.

Brian was married to Dr. Dawn Marie Schmidt, a Registered Pharmacist currently working at Shands Hospital at the University of Florida, on August 10, 2003, in Madison, Wisconsin. He has the opportunity to visit often with his brother Sean and his sister-in-law, who also live in Gainesville, and his parents, who still reside in Sarasota.