Picosecond Laser Procedures to Enhance the Efficacy of Tissue Resection

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ABSTRACT

The fundamental goal of this work was to develop an improved surgical modality in tissue, where minimising thermal damage is paramount, using an ultrashort pulse picosecond laser. Additionally, an investigation into flexibly delivering such pulses via a hollow core negative curvature fibre, in order to enable future minimally invasive endoscopic procedures, was conducted.

Initially, the analysis of colon tissue resection in a porcine model based on plasma mediated laser ablation (at 1030 nm and 515 nm) using a scanning galvanometer is presented. A minimal thermally damaged region (<60 µm) and the ability to finely tune the depth of ablation using different scanning strategies, pulse repetition rate, pulse energy and laser fluences are demonstrated. These desirable surgical effects on the tissue were confirmed using surface profilometry and histological analysis. The picosecond laser ablation of healthy and cancerous lung tissue in an ovine model was also investigated. It has been observed that the ablation depth of cancerous tissue is approximately equal to half of the ablation depth of healthy tissue using the same laser parameters. This thesis also demonstrates that secondary effects of plasma formation such as shock wave induced mechanical damage, cavitation/gas bubble formation, can occur dependent on the parameters used. An appropriate scanning strategy (where there is little or no overlap between consecutive laser pulses) therefore needs to be implemented to minimise these detrimental effects. A laser scanning methodology (0% and 20% overlap with consecutive pulses) with enhanced reduction in thermal injury is presented using 20 kHz pulse repetition rate, 1030 nm wavelength and 13 J/cm² laser fluence with a maximum ablation rate of 6 (0% Overlap) and 4 (20% overlap) mm³/minute.

The development of novel hollow core microstructured fibres has enabled the potential for delivery of ultrashort pulse picosecond laser radiation throughout the body. Therefore, in this thesis ultrashort laser pulses suitable for precision porcine colon resection were flexibly delivered via a hollow core negative curvature fibre. The fibre was manipulated via multi-axis robotic device to mimic movements expected during a practical surgical procedure. Again, a controllable change in ablation depth and with a minimum thermally damage region (< 85 µm) is observed. Furthermore, ablation depths are of comparable scale to that of early stage lesions/polyps in the inner lining of the colon and hence provide a level of control of resection suited to surgical application to thin walled structures such as the bowel.
DEDICATION

“To those special people in my life who support, inspire and uplift me”
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<tbody>
<tr>
<td>USP</td>
<td>Ultrashort Pulse</td>
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<tr>
<td>ps</td>
<td>Picosecond</td>
</tr>
<tr>
<td>fs</td>
<td>Femtosecond</td>
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<tr>
<td>ns</td>
<td>Nanosecond</td>
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<tr>
<td>µs</td>
<td>Microsecond</td>
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<tr>
<td>NIR</td>
<td>Near Infrared</td>
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<tr>
<td>3D</td>
<td>Three Dimensional</td>
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<tr>
<td>2D</td>
<td>Two Dimensional</td>
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<tr>
<td>CW</td>
<td>Continuous Wave</td>
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<tr>
<td>PDT</td>
<td>Photodynamic Therapy</td>
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<tr>
<td>PTT</td>
<td>Photothermal Therapy</td>
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<tr>
<td>LASIK</td>
<td>Laser-assisted in situ Keratomileusis</td>
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<tr>
<td>MEMS</td>
<td>Micro-Electro-Mechanical Systems</td>
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<tr>
<td>HC-NCF</td>
<td>Hollow Core Negative Curvature Fibre</td>
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<tr>
<td>ARROW</td>
<td>Anti-Resonant Reflecting Optical Waveguide</td>
</tr>
<tr>
<td>PCF</td>
<td>Photonic Crystal Fibre</td>
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<tr>
<td>PBF</td>
<td>Photonic Bandgap Fibre</td>
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<tr>
<td>MFD</td>
<td>Mode Field Diameter</td>
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<tr>
<td>NA</td>
<td>Numerical Aperture</td>
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<tr>
<td>AOM</td>
<td>Acousto Optic Modulator</td>
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<tr>
<td>FP</td>
<td>Fabry-Perot</td>
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<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
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<tr>
<td>CPD</td>
<td>Critical Point Drying</td>
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<tr>
<td>OPA</td>
<td>Ovine Pulmonary Adenocarcinoma</td>
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<tr>
<td>JSRV</td>
<td>Jaagsiekte Sheep Retrovirus</td>
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<tr>
<td>CCD</td>
<td>Charge Coupled Device</td>
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<tr>
<td>Er: YAG</td>
<td>Erbium- Yttrium Aluminium Garnet</td>
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<td>Ho: YAG</td>
<td>Holmium- Yttrium Aluminium Garnet</td>
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<tr>
<td>Nd: YAG</td>
<td>Neodymium- Yttrium Aluminium Garnet</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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Journal article

Syam M.P.C. Mohanan, Rainer J. Beck, Nicholas P. West, Michael Shires, Sarah L. Perry, David G. Jayne, Duncan P. Hand, Jonathan D. Shephard, Preclinical evaluation of porcine colon resection using hollow core negative curvature fibre delivered ultrafast laser pulses, Manuscript submitted to Journal of Biophotonics (Under Review)

Conference Contributions


Syam M. P. C. Mohanan, Rainer J. Beck, Donald Risbridger, Sarah L. Perry, Mike Shires, David Jayne, Duncan P. Hand, Jonathan D. Shephard, Preclinical evaluation of ultrashort pulsed laser surgery, Health innovation and Technologies Festival, Edinburgh, UK, 2019, Poster Presentation.


Donald R. Risbridger, Rainer J. Beck, Syam M. P. C. Mohanan, Jonathan D. Shephard, Aongus McCarthy, Mike Shires, David Jayne, Novel optical technologies for ultrashort pulsed laser surgery’ Conference paper submitted to SPIE/OSA European Conferences on Biomedical Optics (ECBO), 2019, Munich, Germany
“If you really look at it, I was trying to sell a dream”

Dr. Charles K. Kao (1933-2018)
Nobel Laureate in Physics, 2009
Chapter 1
Introduction

1.1. Introduction to lasers in medicine and surgery

The unique characteristics of laser light paved the way to its widespread applications in medicine. The ruby laser was first constructed by Theodore Maiman in 1960, and very shortly after was used to treat the retinal issues using the photocoagulation capability of the laser [1.1-2]. The ruby laser was also used in the area of dermatology [1.3-4] during the same period and these were therefore the earliest reports of how lasers might be used to develop new therapies. In 1968, Mester observed the cellular changes in mice made by a ruby laser of 694 nm wavelength [1.5] and he initiated the photo-biomodulation based research. Several studies have been made to understand the photobiological and photochemical effects of laser irradiation on living organisms, after that researchers realized the intense influence of laser light in medical diagnosis and laser therapy [1.6-7]. The use of lasers in optical diagnosis and laser therapy have been making significant advancements through the analysis of photo-induced light and by-products.

In 1966, the first laser surgery to remove cancer tissue was supervised by Dr. Goldman [1.8] and shortly after this Beckman et al. performed a refractive eye surgery based on pulsed carbon dioxide laser in 1971 [1.9] showing that the field of laser medicine was rapidly expanding. The invention of the CO\textsubscript{2} and Er: YAG laser in 1964 and 1975 respectively stimulated the use of infrared wavelengths in medical surgical research. CO\textsubscript{2} lasers (wavelength of 10.6 \(\mu\text{m}\)) and Er: YAG lasers (wavelength of 2.94 \(\mu\text{m}\)) have been the most commonly used lasers for surgery because of the high absorption by water in the biological cells at these wavelengths, as shown in figure 1.1. The application of CO\textsubscript{2} lasers to general surgery was further developed by Kaplan [1.10-13]. Glaucoma was treated in patients by Pollack et al. in 1976 using another laser (argon ion laser) [1.14], which operates at 514.5 nm. Histology analysis of the iris has been done after the laser iridotomy to analyse the usefulness of the continuous wave (CW) argon ion laser in glaucoma treatment. Penetration to iris was achieved without excessive difficulty compared to conventional technique. Also, Photodynamic Therapy (PDT) has been one of the most significant fields of research in the history of lasers in medical application where cancer research and dermatology are the main application areas of PDT [1.15]. The diode lasers with visible wavelengths are mostly using for PDT application [1.16].
De Mari et al. developed a laser with pulse lengths in the order of a few tens of picoseconds just six years after the invention of the first laser [1.18]. Later, the researchers used picosecond lasers for the medical research. However, successful clinical trials of these ultrashort pulsed lasers have only recently been developed to a significant extent because of the cost, availability and robustness of the laser. Researchers have applied the capability of picosecond lasers to readily induce optical breakdown in eye surgery. The application of plasma induced effects in eye surgery was initially reported by Krasnov [1.19] and Aron-Rosa et al. [1.20] in 1977 and 1981 respectively.

1.2. Motivation – Ultrashort pulsed picosecond laser and hollow core negative curvature fibre for colon resection

The early use of long pulsed lasers (sub-microsecond or longer) for endoscopic surgery, in this case the treatment of gastric tumours, was also reported during the 1980’s [1.21-22]. Surgeons performed experimental studies on the bowel tissue of animals to assess and compare the thermal damage mechanism and haemostasis ability of long-pulsed laser scalpels and electrocautery tools [1.23]. They concluded that the thermal necrosis induced by these surgical modalities are similar in nature. It is unsurprising therefore that established electrocautery based surgical procedures have continued as the standard method for colonic polypectomy and colon tumour resection because the long pulsed laser has no significant improvement in reducing thermal damage. Consequently, the uptake of lasers for general high precision surgical procedures has been limited.
The work in this thesis aims to address the limited uptake of lasers into surgical procedures by developing a new surgical process based on ultrashort picosecond lasers to investigate how such systems might offer a significant improvement in the precision of resection and a large reduction in collateral thermal tissue damage compared to existing electrocautery tools and previously reported laser systems. In order to do this, picosecond (ps) laser ablation via a scanning galvanometer on a porcine colon and sheep lung tissue model was investigated. A negligible thermally damaged surgical zone and fine depth control of tissue resection has been analysed with the help of optimized laser pulse energy, laser fluence, pulse repetition rate (PRR) and pulse overlap or scanning strategy. The main aim of this work was to check the feasibility of using a ps laser to create negligible thermal and mechanical damage on colon tissue. This is a key finding because the surgical procedure in endoluminal gastroenterology requires high precision with minimal necrotic tissue to minimize the risk of bowel perforation. It is further observed that such pulses (6 ps) suitable for precision porcine/murine colon resection can be flexibly delivered via a hollow core negative curvature fibre (HC-NCF) opening up the possibility to develop minimally invasive, endoscopic surgical procedures.

Until recently, the implementation of ultrashort pulsed lasers in endoscopic or colonoscopic surgery was impossible because of the lack of a suitable delivery optical fibre. Although silica is an ideal bio-inert material for clinical use, the low damage threshold (~ 4 J/cm² at 6 ps pulse width [1.24]) and high non-linearity prevents conventional solid core silica fibres from carrying these high energy laser pulses. Recently, the delivery of high peak power 1030 (up to 92 µJ pulse energy with a core diameter of ~38 µm) and 515 nm picosecond lasers via 8 cell silica hollow core negative curvature fibres (HC-NCF) has been demonstrated [1.25-26]. These fibres guide via the anti-resonant reflecting optical waveguide (ARROW) mechanism [1.27]. The role of hollow core negative curvature fibre (HC-NCF) in industrial and medical applications has also been recently reported [1.28]. The experiments reported in this thesis were designed to investigate the pre-clinical evaluation of a ps laser in colon/lung tissue resection. Preliminary studies on colon/lung tissue resection based on ultrafast laser pulses delivered via a galvanometer have been reported recently [1.29-30]. Researchers have used multicore [1.31] and graded index multimode [1.32] fibres for the delivery of ultrashort pulses to demonstrate the laser ablation. Additionally, prior work has established porcine tissue as an effective medical model for several studies related to human diseases [1.33] and is consequently applied in porcine vocal fold tissue resection using 1.5 ps laser pulses.
delivered through kagome lattice hollow core photonic crystal fibre for microsurgery [1.34]. The microsurgery laser ablation on the vocal fold tissue has a depth and width of ablation of around 43 and 40 µm respectively at a pulse energy of 1.2 µJ. In this work, pulse energies of 28 µJ were successfully delivered to the porcine/mouse colon surface without any damage to the fibre. Using such pulses with a scanning modality the ablated zone width is approximately 25 times that reported in the vocal fold microsurgery work described above making realistic surgical times a possibility. The aim is to be able to resect early stage lesions/ polyps from the inner lining of the colon tissue by adopting a scanning strategy tailored to the size of the lesion.

The high peak intensity (>10$^{11}$ W/cm$^2$) of the picosecond laser at the tissue surface means that the light is strongly absorbed via non-linear processes, generating a plasma, a bright white light plume. Once initiated the plasma absorbs the rest of the laser energy and efficaciously couples the energy to the tissue to create a non-thermal surgical effect and hence tissue resection with precise crater size, shape, depth and minimal thermal damage [1.35]. The plasma mediated tissue ablation was investigated and discussed in [1.36-37].

The porcine colon tissue has been taken as an experimental sample to check the efficacy of picosecond laser in tissue resection. The ovine lung tissue and murine colon was also used for the ablation studies. This thesis presents the results of experimental work on the colon/lung tissue using the picosecond laser (based on galvo and HC-NCF delivered pulses) and the outcome of the development of a flexible hollow core fibre optic delivery system for picosecond laser transmission. In doing this, a promising route towards implementing ps laser surgery in a minimally invasive modality via HC-NCF has been investigated. The objective of this study is the resection of tissue at the mm$^3$ volume level and is particularly relevant for the scale of early stage colonic tumours. To develop an optical fibre based tissue resection system is also under the scope of work.

Key measurable objectives of this work are as follows:

- The ablation depth of the laser surgical zone is a key factor in determining the efficiency of laser resection. In order to measure this ablation depth an Alicona Infinite focus profilometer was used. The effect of laser parameters such as pulse energy, pulse overlap, pulse repletion rate and iterations of the scan pattern on the measured ablation depth were investigated.
The ablation rate of the process is another important factor to assess the ability of laser surgery as this ultimately dictates surgical time. The ablation rate is defined as the ratio of volume of the ablated crater to the time taken for ablation.

The width of the thermally damaged area on the laser surgical zone is paramount when considering post-surgery complications in colon surgery. Therefore analysis of histology samples using ImageScope software was used to determine the relation of the width of thermal damage on laser processing parameters.

The ability of HC-NCF to carry ultrashort laser pulses (optimised for tissue ablation) without any change in spectral and temporal shape was assessed. The spectral and temporal quality of the pulse was measured using a spectrometer and an autocorrelator.

The optimization of coupling efficiency from the laser to HC-NCF was also investigated in order to maximise the power that could be flexibly delivered to the tissue surface.

1.3. Overview of Thesis

This thesis is organised into the following chapters:

Chapter 2 gives an introduction to the conventional surgical tools and modalities, laser tissue interaction mechanisms, ultrashort pulsed laser induced plasma mediated resection and the effect of secondary events of plasma formation in tissue ablation. The morphology of colon and lung tissue in animal models used for the experimental work and the evolution of hollow core negative curvature fibre (HC-NCF) used for the surgical application are also described in this chapter.

Chapter 3 presents an in-depth investigation of ultrashort pulsed picosecond laser (1030 nm) ablation in colon tissue of a porcine model. The laser pulses are delivered through a galvanometer scanhead to test the efficacy of different pulse overlaps in laser surgery. Various laser parameters (pulse energy, pulse repetition rate (PRR) and laser fluence) are applied to the tissue to assess the change in ablation depth, ablation rate and thermal damage. This chapter also describes the methods to avert the secondary effects of plasma formation such as shock waves and cavitation bubbles (gas bubble). The analysis of laser
Ablated tissue has been carried out using three dimensional (3D) profilometry and histology results.

Chapter 4 describes the efficacy of 515 nm wavelength ultrashort pulsed picosecond laser in colon tissue surgery. Using a reduced focused spot diameter enabled the testing of higher laser fluences and low pulse energies on the colon tissue compared to the experiments presented in chapter 3 (at 1030 nm). Different pulse energies, pulse overlap, pulse repetition rates and laser fluences are tested for resection. The importance of ultrashort pulsed picosecond laser in colon tissue surgery has been analysed with the assessment of shape, depth and thermal damage to the surgical zone.

Chapter 5 provides the basic understanding of 515 nm picosecond laser ablation in healthy and cancerous lung tissue in an ovine model. This pilot study gives an insight to the possibility of ultrashort pulsed picosecond laser in cancer surgery. The change in ablation depth in cancerous and healthy lung tissue according to different laser fluences and repetition rates are also presented in this chapter.

Chapter 6 presents the characterisation of hollow core negative curvature fibre delivered picosecond laser pulses and its application in colon resection on a porcine and mouse model. The coupling methodology of picosecond laser light into the optical fibre and power coupling efficiency of the fibre for various pulse widths are also illustrated in this chapter. Different pulse energies, scanning strategies and laser fluences are tested on the tissue via HC-NCF delivered laser pulses. This chapter demonstrates a way towards establishing the picosecond laser resection in minimally invasive surgical modality.

Chapter 7 discusses the the conclusions derived from this work and scope of future works associated with the experiments and results presented in this thesis.

1.4. References


[1.5] E. Mester, B. Szende, and P. Gartner, [The effect of laser beams on the growth of hair in mice], Radiobiol Radiother (Berl), 9, 621-626, (1968)


imaging through a multimode fiber, Biomedical Optics Express, 10, 423-433, (2019)


Chapter 2

Background

2.1. Conventional Surgical tools and methods

In this first section the review is confined to established surgical tools and techniques. It is interesting to note that the topic of tissue resection, or surgery, has a long history and there is evidence that it was started at the Stone Age [2.1]. The initial concept of a surgical blade was first proposed by Hippocrates [2.2]. Much later, in the early 20th century Morgan Parker invented the modern form of the blade and handle surgical scalpel [2.3]. Additionally, electrosurgical devices, another form of tissue resection tool are now well established. The use of electricity in medicine first started in the 18th century [2.4] however, the modern form of the electrosurgical device was developed by Bovie and Cushing where Dr Cushing first demonstrated the use of such a device for a surgical application in 1926 [2.5]. Finally, in 1966 Dr. Goldman supervised the first laser surgery [2.6], long pulsed laser scalpels have been used in clinical practice which are introduced in section 2.1.4 and discussed in more detail later in this chapter.

2.1.1. The Scalpel

The scalpel blade instrument is the most consistently used tool of surgeons. It is composed of a blade and a scalpel handle. Scalpels are used to make incisions in the skin or other deeper tissue when a fine and precise cut is required for the operation. These blades are also used to separate and dissect various types of tissue. The two most common blades used for surgical operations are No. 10 and No.15 blades [2.7-8]. The No. 10 blade is one of the largest scalpel blades (Approx. 7.1 mm) used to make large incisions and the No.15 blade (Approx. 4.1 mm) is for smaller incisions typically used for facial surgery. The width of the cut is dependent on the sharp part of the blade and the damage to surrounding tissue is negligible but the application of a scalpel in highly vascular areas is ‘restricted’ as haemostasis is paramount [2.9]. Different types of scalpel blades and scalpel handle are shown in below figures 2.1 and 2.2. Careful planning and skilled execution is required for a high quality surgical incision. From a surgeon’s perspective bleeding is the main issue during scalpel blade based surgery as it can lead to an unclear vision of the operating field, this has the significant impact of increasing the surgery time. Another major drawback of this surgical method is post-operative pain and post-surgical scarring [2.10-11].
2.1.2. Harmonic Scalpel

The harmonic scalpel is composed of an active bladed device, in contrast to a traditional passive scalpel blade. It consists of an active blade, a power generator, a foot pedal, a transducer and a hand-piece. In the harmonic scalpel, the power to the harmonic hand-piece is delivered by a generator [2.14]. The transducer converts the electrical energy to ultrasonic vibrations which cause the active blade to expand and contract as the wave passes along the entire length of the device. This movement of the active blade system is against the inactive or stationary part of the blade system as shown in the figure 2.3. Also, in the harmonic scalpel, low frequency ultrasonic energy at high power can be used to create frictional heating delivering haemostatic coagulation and/or cutting through cell disruption. Ultrasonic coagulation is due to friction between the active part of the blade and the cells or tissue. This mechanical friction breaks hydrogen bonds to denature protein and generate a sticky protein, coagulum, which is capable of sealing small vessels up to 5 mm diameter [2.15]. The protein denaturisation facilitates coagulation and the cutting up of small vessels.
The harmonic scalpel has advantages over electrocauterization (described below), as it generates less smoke and hence doesn’t obscure the surgeon’s sight of the procedure. Also, the harmonic scalpel can provide more precision in tissue cutting and produces less lateral damage in comparison with electrocauterization. The use of harmonic scalpel in haemorrhoidectomy creates lateral thermal damage up to a maximum of 1.5 mm. The use of electrocautery in porcine small bowel surgery creates damage from hundreds of microns to a maximum of 15 mm [2.17-18]. This is because the harmonic scalpel performs the cutting operation using ultrasonic vibrations whereas electrocautery performs the surgery using a thermal process. The coagulation in harmonic scalpel surgery occurs due to protein denaturisation. The applied mechanical energy is enough to break hydrogen bonds by the generation of heat from intracellular friction that results from high frequency vibration of tissue. The biggest advantage of the harmonic scalpel over traditional blade scalpel is the curtailed bleeding and post-operative wound healing time.

2.1.3. Electrosurgery

Electrocauterization is a surgical modality to excise unwanted tissue and is also used to enable coagulation through sealing of blood vessels. The electrocautery was introduced in surgery to overcome the limitations of surgical blade, such as a) very poor ability for haemostasis b) long time of operation c) the need for the use of sutures in surgical site [2.19]. Electrosurgery uses electricity to produce high frequency current that stimulates water molecules in the tissue to the level of boiling point. Electrosurgery typically utilises
the radiofrequency range of 100 Hz to 5 MHz [2.20]. The electrosurgical generator is capable of producing different current waveforms via modulation. Cutting current, coagulating current and blending current are three important currents produced by an electrosurgical generator, shown in figure 2.4. There are two types of electrosurgical methods (Unipolar and Bipolar) available to excise tissue based on the number of electrodes used at the surgical site [2.21].

![Image of cutting current, coagulating current, and blending current waveforms]

Figure 2.4. The cutting current (a), the coagulating current (b), the blending current (c) [2.22]

### 2.1.3.1. Cutting Current

Cutting current is an unmodulated and continuous current from the electrosurgical generator. In the cutting current waveform the duty cycle is 100% i.e. the current is ON for the full time of operation. The electrosurgical cutting is a non-contact activity in which the electric spark generated in between the active electrode and tissue produces heat and that enables vaporization of tissue.
2.1.3.2. Coagulating current

In electrosurgery the coagulation or blood clotting can be achieved in two ways such as fulguration and desiccation. In the coagulation mode the cutting current is ON for 6% time and OFF for 94% of time cycle. Fulguration is superficial coagulation and it is a non-contact mode of coagulation. The surgeons spray electric sparks to the tissue which results in coagulation and it is a good technique to avoid charring. Deep coagulation is known as desiccation and it is a contact mode coagulation. It causes deep coagulation necrosis and a sticking eschar, shown in figure 2.5. The extension of thermal damage is very high in this type of electrosurgical device with deep coagulation. All the control is in surgeon’s hand because it is a contact mode application and a foot pedal is often use to control the current. Therefore the extent of thermal damage is entirely dependent on the surgeon’s skill with little feedback given to the operator.

Figure 2.5. Electrocautery eschar in rectal surgery [2.23]

2.1.3.4. Blending Current

This technique permits the surgeon to cut and coagulate the tissue at the same time which is a significant advantage over the traditional scalpel. The blend mode is achieved by changing the duty cycle of the cutting current as shown in the figure 2.4c.
### 2.1.3.5. Unipolar and Bipolar Electrosurgery

In unipolar electrosurgery an electrode pad is attached to the patient body and the current flows from the single active electrode (the surgical instrument or blade) through the body to the attached electrode pad and completes the electric circuit [2.24], shown in figure 2.6a. A bipolar electrosurgery unit is composed of a hand piece with two metal electrodes placed like forceps. One electrode performs the operation of the active electrode while the other performs the passive action to complete the current flow through a limited part of the surgical area (tissue) [2.25], shown in figure 2.6b. The bipolar electrosurgery can be used with patients who have done prosthesis but the unipolar electrosurgery is not applicable in this case because the electric field will cause damage to body implants.

![Unipolar and Bipolar Electrosurgical Setup](image)

Figure 2.6. The Unipolar and bipolar electrosurgical setup (a and b) the electrosurgical cut on tissue made with different modulation of electricity (c) [2.26]

### 2.1.4. Long pulsed Laser Surgery

#### 2.1.4.1. Laser Scalpel

Lasers have been developed as an alternative surgical tool to the traditional, harmonic and electrocautery blades and devices described above with the aim to improve control and precision. Long pulsed and CW lasers are in included here as conventional, or existing, surgical tools as they have been demonstrated in a number of clinical procedures such as [2.27-28]. It should be highlighted however that the laser-tissue interaction
mechanism for long pulsed lasers is a different mechanism to the ultrashort (picosecond) pulsed laser systems used for the work reported in this thesis. Here, the term long pulsed refers to lasers with pulses in the order sub-microsecond or longer. More detail on the laser-tissue interaction and applications of long pulsed lasers in surgery are given later in section 2.2

A laser can act as a scalpel to make precise incisions or excision in the tissue while leaving the surrounding healthy tissue relatively intact. For long pulsed and CW lasers the wavelength of the laser light is very important in surgery and it determines the depth of penetration of light into the tissue [2.29]. In the simplest form laser absorption within the tissue leads to molecular excitation, which results in a temperature increase in a tissue. This thermal change in the tissue leads to coagulation, vaporization or carbonisation [2.30]. When the temperature reaches the boiling point of water then vaporisation of tissue occurs. However, coagulation necrosis occurs before the tissue reaches the boiling point and can be used for coagulation during surgical procedures. To achieve coagulation the surgeon keeps the laser beam out of focus after the cutting (removal of tissue) procedure [2.31].

Long pulsed and CW lasers have been used for both invasive and non-invasive modes of surgery. The invention of the articulated arm stimulated the use of CO₂ laser in surgery; however, it restricts the surgeon from performing endoscopic (minimally invasive) surgery because of its size [2.32]. The articulated arm is a combination of hard tubes with movable joints and a hand piece. The hand piece contains the focusing optics for the laser beam connected to the articulated arm. Improved flexibility can be achieved by using optical fibre delivery of surgical lasers. The conventional (silica) multimode fibres or single mode fibres perform the function as a delivery system for surgical lasers in the NIR and visible wavelength range. To perform endoscopic laser surgery, endoscopes are used to deliver the optical fibre to the surgical site with the help of a range of medical imaging techniques [2.33]. However, for wavelengths longer than 2 μm, silica optical fibres cannot be used due to the high absorption of the material in that region. Therefore, a range of non-silica solid core fibres and, more recently, hollow core fibres have been designed for surgical lasers operating in the mid to far IR region [2.34]. More recently, research for Er: YAG laser delivery through hollow core silica fibres was initiated by Urich et al. in 2012 [2.35]. They demonstrated that either a hollow core photonic crystal fibre or a negative curvature fibre have a promising possibility in the area of surgical laser delivery.
system. Also new laser surgery techniques have been reported where the transurethral laser ablation is guided by real time magnetic resonance imaging (MRI) [2.36].

2.1.5. Comparison of conventional surgical methods

Table 2.1 illustrates the comparison of conventional surgical tools. In 1999, Boxem et al. carried out a study related to the cost effectiveness of the Nd: YAG laser and electrocautery tool [2.37]. It has been found that the treatment cost of Nd: YAG laser is slightly higher when compared to the treatment cost of electrocautery for a fixed number of patients and days. The cost of the laser is high compared to the cost of an electrocautery device. The haemostasis ability of a scalpel, electrocautery device and CO\textsubscript{2} laser were evaluated by [2.38] in canine skin tissue. They found that the haemostasis ability in all surgical modalities were similar in nature. It has been found that the heat generation in electrocautery and laser surgery methods were also similar in nature. Prakash et al. performed a study to compare post-operative pain related to electrocautery incision and scalpel incision in abdominal surgery [2.39]. The mean blood loss per unit wound area of patients was analysed for electrocautery (6.46±3.94 ml) and scalpel (23.40±15.28 ml). It was concluded that the electrocautery based surgery is safe compared to post-operative pain caused by a scalpel. The use of anaesthesia was analysed by [2.40]. Here an 810 nm diode laser was compared with conventional techniques in orthodontic soft tissue surgery. Topical anaesthesia was applied for the diode laser cases. However, the use of general or local anaesthesia is a must in scalpel surgery and electrosurgery.
<table>
<thead>
<tr>
<th></th>
<th>Scalpel</th>
<th>Electrocautery</th>
<th>Laser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemostasis</td>
<td>No</td>
<td>Excellent</td>
<td>Excellent</td>
</tr>
<tr>
<td>Visibility of Operating field</td>
<td>Poor, Because of blood in the operating field</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Use of Anaesthesia</td>
<td>Yes</td>
<td>Yes</td>
<td>Used in some cases</td>
</tr>
<tr>
<td>Post-operative pain</td>
<td>High</td>
<td>Moderate</td>
<td>Reduced</td>
</tr>
<tr>
<td>Heat Generation</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Prosthesis Complications</td>
<td>No</td>
<td>Yes, in Monopolar surgery</td>
<td>No</td>
</tr>
<tr>
<td>Cost</td>
<td>Least</td>
<td>Moderate</td>
<td>High</td>
</tr>
</tbody>
</table>

Table 2.1. Comparison of three methods for soft tissue excision. Based on research [2.19, 37-40]
2.2. Laser tissue interaction mechanisms

2.2.1. The photochemical effect

When tissue is irradiated with light, the molecular chromophores in the tissue absorb the light energy which elevates the molecule to a higher energy level. The molecules in the higher energy level participate in a chemical process [2.41], shown in figure 2.7. Various types of chemical reaction can occur, such as photo destruction of the chromophore, bond breaking, cross linking and oxidation via radical formation. The presence of a photosensitizer in certain cells (e.g. hematoporphyrin derivative) stimulates the photochemical reaction with applied light of an appropriate wavelength. Photo Dynamic Therapy (PDT) is a well-known procedure that exploits these photochemical reactions [2.42] and is shown in figure 2.7.

![Figure 2.7. Mechanism of photodynamic therapy [2.42]](image)

PDT is a technique used to eliminate cancerous or pre-cancerous cells in very early stage condition. A light sensitive drug and a light with a wavelength in the visible spectrum are the main elements of this medical treatment. The light sensitive drug is otherwise known as photosensitizer (PS) and is excited by light with an appropriate wavelength. The excited PS produces radical (Type 2) and reactive oxygen species (Type 1). The penetration depth of light depends on the wavelength. It is not possible to use infrared light for this application because of high light absorption by water in the tissue [2.43]. The disadvantage of PDT is that it can only be used to treat cancer at the superficial level, i.e. not deep in tissue. The efficacy of PDT is related to the targeting of light on the particular cancer tissue, which is restricted in endoscopic gastric PDT treatment because of the size and shape of healthy/tumour tissue at the treatment site [2.44]. Also,
photosensitizer used for PDT procedures can make patients sensitive to light for some weeks after the procedure and the photosensitiser has associated toxicity and allergy related complications.

2.2.2. The photothermal effect

Laser absorption by tissue leads to molecular excitation, which is the reason for heat deposition. This heat deposition leads to photocoagulation and vaporization and stimulates the denaturisation of proteins and enzymes. As a result of photocoagulation, the heated region changes colour and loses its mechanical integrity known as coagulated necrosis [2.45]. The photocoagulation is able to stop bleeding during surgery because the damaged cells initiate blood clotting and seal off the blood vessels. When the temperature reaches the boiling point of water within the tissue it boils and evaporates. This process leads to laser thermal ablation and any excess heat can lead to carbonisation and melting of tissue. Table 2.2 elaborates on the temperature dependence of photothermal laser tissue interaction.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Molecular and tissue reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>42-45 °C</td>
<td>Hyperthermia, Hydrogen bond breaking</td>
</tr>
<tr>
<td>45-50 °C</td>
<td>Enzyme inactivation, changes in membrane permeabilisation</td>
</tr>
<tr>
<td>50-60 °C</td>
<td>Coagulation, Protein Denaturisation</td>
</tr>
<tr>
<td>~80 °C</td>
<td>Collagen Denaturisation</td>
</tr>
<tr>
<td>80-100 °C</td>
<td>Dehydration</td>
</tr>
<tr>
<td>&gt;100 °C</td>
<td>Boiling, Steaming</td>
</tr>
<tr>
<td>100-300 °C</td>
<td>Vaporisation, Tissue Ablation, Carbonisation</td>
</tr>
<tr>
<td>&gt;300 °C</td>
<td>Melting</td>
</tr>
</tbody>
</table>

Table 2.2. Laser-Tissue interactions: Photothermal effects [2.45]

Photothermal therapy (PTT) is another treatment for many medical conditions and it is an extension of PDT. In PTT a photosensitizer is excited with a specific wavelength of light and the excited molecules release heat which kills the targeted cells [2.46]. The pulse duration plays a vital role to prevent the thermal damage to surrounding tissue in pulsed laser ablation. To reduce the thermal damage to surrounding tissue the pulse width must
be smaller than the thermal relaxation time. Thermal relaxation time is the time necessary to cool down (Up to 37%) the irradiated tissue via transferring the heat to the surrounding tissue through thermal diffusion. This is an important aspect to consider for both effective coagulation and precise cutting. When the optical penetration depth is less than the laser spot size then the thermal relaxation time $\tau_{th}$ is [2.47],

$$\tau_{th} = \frac{\delta^2}{4\alpha} \quad (2.1)$$

$\delta$ = Optical penetration depth $\quad \alpha$ = Thermal diffusivity

### 2.2.2.1. Thermal Properties of a Tissue

Hyperthermia, coagulation and other irreversible tissue effects can be observed when the tissue temperature increases from the normal body temperature level of around 37 °C, shown in figure 2.8. The first effect is hyperthermia and the temperature above the normal cell temperature leads to this effect typically in the range of 40-50 °C. Hyperthermia therapy is widely used to kill cancerous cells [2.48] and this method is effective to break some molecular bonds and alteration of cell membranes. There are a lot of limitations associated with this treatment, to keep the tumour tissue at a particular temperature without affecting the adjacent healthy tissue is one of the main disadvantages of this treatment modality. Other side effects can be nausea, vomiting, and diarrhoea [2.49].

The effect of coagulation can be observed as tissue temperature is increased to around 60 °C which also leads to coagulated necrosis. In coagulation the temperature damages the tissue proteins with an irreversible state change. This method is used to seal off the blood vessels during surgery. Vaporization of water occurs at 100 °C. The thermal relaxation time of a tissue plays a vital role in the laser tissue ablation process [2.50]. The optical penetration depth is denoted as $L$,

$$L = \sqrt{4k\tau_{therm}} \quad (2.2)$$

Where $\tau_{therm}$ is the thermal relaxation time, $k$ is a measure of the thermal susceptibility of the tissue. If the laser pulse duration, $\tau$, is less than, $\tau_{therm}$, then the heat will not diffuse through the depth of optical penetration. This results a reduction in adjacent tissue damage. On the other hand if, $\tau$, is greater than, $\tau_{therm}$, then the heat will diffuse twice the depth of optical penetration [2.51]. A longer pulse duration is one of the main reasons for
adjacent tissue damage during laser surgery. It is important to minimize the thermal damage to the adjacent tissue by selecting appropriately short pulse durations. Consequently, this is why picosecond (and femtosecond) pulsed lasers are particularly attractive for precision laser surgery although the effectiveness and precision is enhanced when working in plasma mediated ablation regime as discussed later in section 2.2.3 and 2.3.

As the temperature is increased further eventually carbonisation occurs. As a result of carbonisation the adjacent tissue are blackened and smoke is generated during the process. Carbonisation occurs in a temperature range of 100 °C to 300 °C and is associated with laser tissue ablation. As the temperature of the tissue is increased beyond 300 °C melting occurs.

![Figure 2.8. Thermal effects inside a biological tissue](image)

**2.2.2.2. Application of thermal laser processes in tissue resection and coagulation**

The application of long pulsed (0.5 ms) ruby laser was first demonstrated by Zaret et al in a rabbit eye in 1961 [2.52]. Later, the laser treatment on human eye was reported by Campbell *et al.* [2.53] and Zweng *et al.* [2.54] in 1963 and 1964 respectively. After the invention of CO$_2$ laser Beckman *et al.* performed the sclerostomies [2.55] to treat glaucoma using laser pulses with a pulse width from microsecond to CW. In 1977, Stafl *et al.* used CO$_2$ laser to manage the cervical and vaginal intraepithelial neoplasia [2.56]. They found that the CO$_2$ laser was an alternative to the electrocautery and cryst surgery used for the treatment of vaginal intraepithelial neoplasia. They have performed CO$_2$ laser surgery in around 50 patients with different stages of neoplasia and the treatment fails in
10% of patients. During that period, Wright et al. claimed that long pulsed (µs to ms) CO₂ laser surgery is the best surgical modality for cervical intraepithelial neoplasia when compared to the conventional surgical modalities [2.57]. Different lasers were used for the urology related surgical treatments [2.58-60] with pulse width of µs range and CW mode. The Er: YAG laser was used for brain tissue studies in 1997 [2.61] with 250 µs long pulses. They observed thermal and mechanical damage to the ablated tissue. Ho: YAG and Er:YAG lasers were used for orthopaedic surgery [2.28, 62-63] and the researchers found that these lasers were promising tools for this surgery.

A pulsed laser with 0.5 to 2 s pulse width has been used to treat the gastric tumours in late 80’s [2.64-65]. Schroder et al conducted a comparative study in colon tissue ablation using electrocautery, CO₂ laser and Nd: YAG laser with a 2s pulse width. It was found that the thermal damage created by all three surgical tools are approximately half a millimetre in width [2.66], This can then be the cause for further complications in colon surgery.

In 1997 Hohenleutner et al. conducted an in-vitro model of Er: YAG laser ablation on human skin samples to evaluate the ablation rate and thermal necrosis according to the change in laser fluences and number of pulses [2.67]. An in vivo study was also conducted to optimize the laser irradiation parameters for an effective surgical modality. An Er: YAG laser with a pulse energy of 100 to 500 mJ (in 50 mJ steps), pulse width of 250 µs and pulse repetition rate of 7 Hz were used for laser ablation. The operator could achieve a spot size of 3 and 4 mm by changing the hand piece of the laser system. Healthy human skin was utilized for this study and fresh samples were kept in 0.9% of NaCl solution. The samples were removed from the solution two hours before the ablation procedure. The histological and microscopic examinations were performed on the sample to find out ablation depth, width of thermal necrosis and ablation rate. It was observed that tissue ablation was effective with a 500 mJ pulse energy and a 4 mm spot size. Twenty-four samples were treated and evaluated for the ablation rate and thermal necrosis by increasing the number of pulses from 5 to 40. The 3 mm hand piece was also used for this treatment modality. The detected ablation threshold is 1.65 J/cm² with a 200 mJ pulse energy and 4 mm spot size. The maximum thermal damage observed was approximately 75 µm when applying 40 pulses, they also confirmed that the width of thermal necrosis is increasing with the number of pulses on the same spot. The spot size of the laser beam used for this ablation was 3 mm hence limiting the absolute precision to this size regime.
With this relatively large spot there is a resultant effect of high thermal damage when increasing the number of pulses further. The ablation rate graph is shown in figure 2.9.

![Ablation rate graph](image)

Figure 2.9. Ablation per pulses Vs laser fluence [2.67]

The application of Er: YAG and Ho: YAG laser in the urology field provided solutions for many complications related to urethra, bladder and other organs related to urology. In 2005 Varkarakis et al. incised the healthy urethra and bladder neck tissue of a group of 18 pigs with Er:YAG (Group of 9 pigs) and Ho:YAG (Group of 9 pigs) lasers using an in vivo animal model (Cystoscope) to evaluate differences in wound healing and scar formation [2.68]. The lasers were used to make three 1 cm long incisions, one in the mid urethra and two at the bladder neck. The histological and quantitative analysis were performed on the sample tissue of urethra, bladder neck and three animals were killed in each group on post-operative days 0, 6 and 14. The Er: YAG laser had a pulse width of 70 µs, pulse energy of 20 mJ and repetition rate of 10 Hz and the Ho: YAG laser had a 300 µs pulse duration, pulse energy of 500 mJ and repetition rate of 3 Hz. In the Er: YAG and Ho: YAG laser systems the light was transmitted through a 250 µm core, 2 m long sapphire fibre and 300 µm core, 3 m long silica fibre respectively.
The width of the granulation zone and incision depth were considered in the ablated section of pig’s urethra and bladder and the changes were noted according to post-operative days 0, 6 and 14. The collateral thermal damage caused by the Er: YAG laser is very small when compared to the collateral thermal damage of the Ho: YAG laser ablation as seen in figure 2.10. The collateral thermal damage is determined by the width of the granulation tissue at the base of the wound. It was observed that the width of granulation tissue at the 6th & 14th post-operative days in each group of pigs was 900±100 & 430±100 µm for the Er: YAG laser and 2280±700 & 1580±250 µm for the Ho: YAG laser. Additionally, the incision by the Er: YAG laser was almost healed at the post-operative day 14 when compared to the incision by the Ho: YAG laser as seen in figure 2.10.

The comparison of ablation rate of vitreous tissue (from the eye) and distilled water was performed by M. Krause et al. in 1999 [2.69]. An Er: YAG laser was used for this application with a repetition rate of 1Hz and pulse widths of 140, 190 and 240 µs. The selected pulse energies were 25, 35, 45, 75 and 100mJ. Fresh porcine eyes were used for this experiment and the vitreous part was removed from the eyes using anatomic forceps and separated with surgical scissors. The vitreous part was immediately transferred into a test tube. The distilled water was filled into an identical test tube for the laser ablation experiments. The beam diameter at the sample surface was 2mm. The changes in the weight of both vitreous and water were measured before and after laser ablation to calculate the ablation rate of the laser system. It was shown that the ablation properties of both vitreous and water were similar but not an equal rate i.e. the ablation rate per pulse
(µg/µs) of vitreous tissues were 3 µg to 45.8 µg (at 240 µs), 10.4 µg to 53.8 µg (at 190 µs) and 17.9 µg to 24.2 µg (at 140 µs). On the other hand the ablation rates in distilled water were 11.7 µg to 49.3 µg (at 240 µs), 14.4 µg to 55.7 µg (at 190 µs) and 20 µg to 25.3 µg (at 140 µs) according to pulse energies of 25, 35, 45, 75 and 100mJ. It was also possible to establish reproducible and constant ablation rates in both samples in each of 10 sequential series of 50 laser pulses and the amount of ablation in both samples with a single laser pulse shows a linear increase with increasing pulse energies and decreasing pulse lengths.

The design and construction of hollow core polymer fibre lined with an omnidirectional mirror was first reported by Burak et al. in 2002 [2.70]. The light confinement is achieved by the various submicrometer thick layer of glass and low refractive index polymer in the inner side of the hollow core fibre arranged in an alternative manner. The wavelength is scaled by 0.75 to 10.6 µm with a transmission loss of 1 dB/m at 10.6 µm wavelength. It was observed that the maximum laser power density coupled through this fibre without any damage to the fibre was 300 W/cm². Dr. Jamie Kaufman performed the first minimally invasive laryngeal CO₂ laser surgery on a human [2.71]. The polymeric photonic bandgap fibre used in this procedure was based on the work done by Burak et al.

In 2011 Duncan et al. performed an investigation to evaluate the depth of thermal damage caused by harmonic scalpel and CO₂ laser on a cadaveric tongue [2.72]. Two cadaver heads were used for the study. Three incisions were created with a harmonic scalpel of 5 W power and five incisions made by a CO₂ laser with power settings of 13, 16 and 18 W. The samples were evaluated by histopathological examination. The harmonic scalpel and CO₂ laser produced a mean thermal damage of 0.69 and 0.30 mm respectively [Table 2.3]. It is also observed that the harmonic scalpel is an efficient tool to coagulate larger diameter blood vessels compared to the laser. The CO₂ laser can coagulate blood vessels of diameter up to 2 mm but the harmonic scalpel can coagulate blood vessels of diameter up to 5 mm. In conclusion, the CO₂ laser is an efficient tool when compared to the harmonic scalpel according to the capability to produce less lateral thermal damage on adjacent tissue.
<table>
<thead>
<tr>
<th>Thermal damage depth (mm)</th>
<th>Method</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>Harmonic Scalpel</td>
<td>0.69</td>
<td>0.16</td>
</tr>
<tr>
<td>0.82</td>
<td>Harmonic Scalpel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.51</td>
<td>Harmonic Scalpel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.22</td>
<td>CO2 Laser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>CO2 Laser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.43</td>
<td>CO2 Laser</td>
<td>0.30</td>
<td>0.08</td>
</tr>
<tr>
<td>0.31</td>
<td>CO2 Laser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.28</td>
<td>CO2 Laser</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3. Thermal damage depth by harmonic scalpel Vs CO2 Laser [2.72].

The lack of an ideal optical fibre delivery system remains a serious hurdle to surgical applications of Er: YAG laser. However Er: YAG lasers have been used for some surgical applications with a range of optical fibre solutions [2.73-77]. None of these fibre delivery systems for Er:YAG are considered as promising as the Negative Curvature Fibre (NCF) or Hollow Core Photonic Crystal Fibre (HC-PCF) used by Urich et al. [2.78] for the ablation conducted in a porcine model. The aim of this study was to demonstrate that the laser delivered through a HC-PCF has sufficient energy to excise soft and hard tissue. Fused silica is used to fabricate the HC-PCF fibre by using a conventional stack and draw technique described in a report by Yu et al. [2.79] and the structure of the fibre is shown in figure 2.11.
Figure 2.11. SEM picture of the negative curvature fibre. $D$ is the core diameter, $t$ is the inner wall thickness and $r$ is the radius of the bent shape [2.78]

An Er: YAG laser (Impex High Tech ERB 15) of wavelength 2937 nm, pulse width of 225 µs and pulse repetition rate of 15 Hz was used for this application. A lens was used to couple the Er: YAG laser to the HC-PCF fibre core of 94 µm and the coupling efficiency achieved was 35% because of the mismatch of laser and fibre mode profile. The fibre end was encapsulated with a sapphire end tip and the sapphire window was attached in the fibre by using a heat shrinking sapphire tube as seen in figure 2.12.

![Figure 2.12. Endtip mounted onto the fibre using a heat shrinking tube [2.78]](image)

The output energy in the fibre tip was 30 mJ with a fluence $> 500$J/cm$^2$ and is sufficient to ablate the porcine bone and muscle tissue as seen in figure 2.13. However, no histology was carried out so the extent of the thermally damaged region cannot be assessed. Also, issues in terms of coupling the laser into the fibre and developing a robust fibre end probe
need to be solved in order to achieve the use of Er: YAG laser delivered through negative curvature HC-PCF fibre in endoscopic and normal surgical procedures.

Figure 2.13. (a) pig bone; (b) a hole in the bone showing ablation depth of 265 µm; (c) ablation in porcine muscle; (d) The ablation on porcine muscle surface with different laser shots [2.78].

2.2.3. Plasma Mediated Ablation

When a laser interacts with solids or fluids there is a possibility of plasma formation if the power density exceeds $10^{11}$ W/cm$^2$ [2.80]. Clean and accurate removal of tissue can be achieved through plasma induced ablation with the help of appropriate laser parameters. The local electric field, $E$, is the most important parameter in plasma formation. If the applied electric field is high enough then it causes the ionization of molecules and atoms resulting in optical breakdown in the tissue [2.51]. The equation for local power density, $I$, in basic electrodynamics is given as,

$$I(r, z, t) = \frac{1}{2} \epsilon_0 C E^2$$  \hspace{1cm} (2.3)

Where $\epsilon_0$ the dielectric constant and $C$ is the speed of light. The threshold power density for optical breakdown is $\sim 10^{11}$ W/cm$^2$, thus the plasma ionization occurs. Commonly, the plasma generation due to a high intensity electric field is known as dielectric breakdown. The ultraviolet, visible and infrared wavelengths are strongly absorbed by plasma to stimulate the laser ablation using plasma energy.
2.2.3.1 Plasma generation in biological tissues

Figure 2.14. The process of plasma formation and associated processes [2.81]

The high intensity laser pulses incident on the tissue surface creates high electric field on the target surface. This process leads to multiphoton ionization. Multiphoton ionization is a process in which the high intensity photons excite the bound electrons to the higher energy levels. The rate of multiphoton ionization is \( I^k \), where \( I \) is the irradiance of the laser and \( k \) is the number of photons needed to excite the electron. In the next step, free electrons absorb the incoming photons and accelerate, this process is known as Inverse Bremsstrahlung Absorption (IBA). As a result of this process the electron acquires enough kinetic energy that surpass the bandgap energy. These electrons collide with atoms to generate another free electron. This process is known as impact ionization. These two low energy free electrons repeat the process of IBA and impact ionization to create more free electrons. As a result of this continued process (avalanche or cascade ionization) an enormous growth in the number of free electrons occurs. When the free electron density surpasses \( 10^{21} \) electrons/cm\(^2\) the optical breakdown occurs with the formation of white intense plasma. The process of plasma generation is depicted in figure 2.14.
Figure 2.15. The plasma formation at the laser focal point during the temporal evolution of a pulse [2.81]

Figure 2.15 shows multiple plasma generation during the pulse with high fluence. At point one (1) in the pulse, the laser induced plasma forms at the focused spot of the laser, at point 2 and 3 the fluence increases during the pulse, which will initiate a series of plasma formation from the beam waist to the incoming laser beam. This moving optical breakdown mechanism was initially derived by Raizer et al. [2.82].

A pulse width of less than 10 ps is normally considered as an ultrashort pulse [2.83]. The process of multiphoton ionization generates the seed electrons for the avalanche process. Ultrashort pulses deposit the energy from the electrons (generated due to avalanche effect) to the lattice before the electron cooling time (~1 ps) passes. This process mainly occurs in the fs regime because the pulse duration is less than 1 ps. In the ultrashort pulse ps regime, the electron cooling time passes but the excited electrons deposit the energy before the lattice heating time passes (> 1ps). The high intensity of the energy breaks the lattice bond before the energy transfers to the adjacent lattice, in this case the tissue matrix is plasmatized as a result of the interaction between ultrashort pulsed laser and tissue [2.84]. Also the thermalisation time of free electrons energy is considered as 10 ps [2.85]. In 1999, Joachim et al. conducted a study in water for the plasma generation using nanosecond to fs laser pulses [2.86].
2.2.4. Photomechanical Interactions

For a photomechanical interaction to occur lasers with pulses in the nanosecond regime or even shorter (picosecond or femtosecond) are required. The electric field of this tightly focused pulse has sufficient peak power to ionize the atoms in the tissue and form a plasma [2.87] which is an ionized region with a high concentration of free electrons and positive ions. This process is known as optical breakdown and laser light with a power density greater than $10^{11} \text{W/cm}^2$ (as discussed in 2.2.3) is required to produce the plasma. This plasma absorbs all the incoming radiation and expands very quickly in the form of mechanical shock waves travelling at supersonic speed. These shock waves result in mechanical disruption of the histological architecture of a tissue also known as photodisruption. The tissue separation occurs due to mechanical energy during photodisruption process. Shock waves travel to the nearby tissue area of optical breakdown region, which limits the precision in ablation at the breakdown zone [2.88]. The dependence of plasma generation and photodisruption to power density is clearly demonstrated in figure 2.16. The ophthalmological area is widely using the photomechanical effect of laser tissue interaction.

Figure 2.16. The outline of laser tissue interaction associated with power density [2.51].
When the plasma is generated on the tissue surface by the interaction of an ultrashort pulse, the locally elevated temperature of the plasma is confined at the focal volume to effectively couple the energy to the sample. The time for thermal diffusion, to transfer the temperature to other parts of the tissue by heat conduction, is extremely short. As a result of the plasma ionization process an effective non-thermal surgical effect can been created at the tissue surface. The secondary effects of optical breakdown or plasma formation are the product of shock wave generation, cavitation bubble formation and jet formation. The high temperature and pressure of the plasma leads to the expansion of the plasma, as a result of this process a shock wave generates (as shown in figure 2.17) and travels at supersonic velocity in the tissue; later, the speed reduces to the speed of sound in water. This shockwave creates mechanical breakdown in the tissue; as a result of this breakdown mechanical damage a cavitation bubble formation occurs because of the pressure of the fluid in the tissue. The additional processes that occur after the optical breakdown is depicted in figure 2.18. The time scale of the occurrence of these different physical process during the interaction of high intensity of laser pulses with tissue is presented in figure 2.19.

The plasma mediated resection of tissue is spatially confined to the plasma formation area. On the other hand, the shock wave cavitation and jet formation propagate out of the plasma formation zone and creates mechanical damage to the adjacent tissue which can be a detrimental effect when considering ultra-high precision surgical resection.

Figure 2.17. Schematic representation of shockwave propagation during plasma mediated ablation
Figure 2.18. The secondary processes associated with the plasma generation [2.51].

Figure 2.19. Time scale of the occurrence of processes involved in the plasma formation. The 2nd represents the second occurrences of the processes. [2.51]
2.2.4.1. Cavitation bubble and jet formation

The rapid expansion of plasma leads to the formation of shockwaves and cavitation bubbles in the tissue. Cavitation bubbles may occur if the optical breakdown occurs inside the tissue [2.51]. The use of laser pulses with high spatial pulse overlap, high pulse energy, high repetition rate and high Rayleigh length can create optical breakdown inside the tissue. Previous investigations [2.89] regarding cavitation bubble used a photographic technique, where, Niemz showed the experimental demonstration of a cavitation bubbles in human cornea using a 30 ps pulse, as shown in figure 2.20. The corneal tissue was fixated immediately after the laser ablation to prevent the bubble collapse. In contrast with Niemz’s argument, in 2008 Pepose et al. described that the generated cavitation bubble collapses after a short lifetime (in the range of µs), as a result which a gas bubble forms in the tissue [2.90]. The figure 2.21 illustrates the process of plasma formation to gas bubble formation. Plasma was generated at the laser focus at stage 1. The shock wave formation due to the expansion of plasma at supersonic velocity is showing at stage 2. When the speed of the plasma is reduced and equal to speed of sound in water (1500 m/s) then a cavitation bubble generates (stage 3), a gas bubble is then formed after the cavitation bubble collapse (stage 4). The lifetime of the laser induced cavitation bubble has been described in [2.89]. Vogel et al. claimed that the lifetime of the cavitation bubble depends on the applied laser pulse energy and close to the optical breakdown regime the bubble life time is less than a microsecond [2.85, 91]. Jet formation is a process that usually occurs at the collapse of the cavitation bubble near to the solid boundary of the tissue sample. The asymmetric collapse of the cavitation bubble leads to the formation of jet inside the tissue to create a tearing effect.

The significant consequence of the cavitation bubble effect in plasma mediated ablation processes with a picosecond laser is that the laser repetition rate and scanning speed must be carefully controlled in order to avoid subsequent incoming laser pulses interacting with expanding or collapsing bubbles. Therefore the pulses need to be sufficiently spatially and temporally separated by the scanning strategy in order to optimise the ablation process. This is experimentally demonstrated in the chapter 3 and 4 of this thesis.
Figure 2.20. Cavitation bubble in a human cornea with pulse width of 30 ps and pulse energy of 1 mJ [2.51]

Figure 2.21. Evolution of gas bubble in a tissue after laser induced optical breakdown. The stages are marked from 1 to 4. 1) plasma formation 2) shock wave generation 3) cavitation bubble 4) gas bubble [2.90]
2.3. Application of ultrashort pulsed laser in tissue resection

The capability to create plasma mediated ablation and effectivity couple the energy to the tissue make ultrashort pulse lasers an ideal tool for surgery of heat sensitive tissue. Plasma induced resection is a non-thermal and nonlinear surgical modality. The ultrashort pulsed (USP) lasers have been using for various types of surgical procedures in ophthalmology [2.92-95]. The use of USP lasers in other medical areas are limited because of the lack of flexible fibre optic probe to actualise the endoscopic procedures. However, research in different medical areas have been reported related to the capability of USP lasers to create precise ablation with negligible thermal damage [2.96-98].

The Laser-Assisted in Situ Keratomileusis (LASIK) surgery based on femtosecond laser is widely accepted surgical procedure. In 2003, Ratkey et al. performed first clinical demonstration [2.99]. The photodisruption property of USP laser has been used for different types of eye surgery such as capsulotomy, corneal transplantation and cataract treatment [2.100-102]. Zysset et al. investigated the picosecond optical breakdown property in a corneal endothelium to assess the efficacy of 40 ps Nd: YAG laser in ophthalmic surgery [2.103]. It was observed that damage less than 100 µm was achieved for an 8 µJ pulse energy, as an ideal parameter for creating surgical incisions in the transparent structure of eye. The femtosecond laser can achieve the photodisruption effect with reduced pulse energy and negligible thermal damage when compared to the picosecond laser. This property makes fs laser an indispensable tool for transparent tissue surgery (eye surgery) [2.104].

Holger Lubatschowski et al. demonstrated the use of a fs laser for refractive eye surgery where a 300 fs titanium sapphire laser was used for this operation [2.105]. The laser focus was scanned to perform a spiral scan pattern on the porcine cornea. A 7 μm laser spot size with 15 μm spot separation was used to demonstrate the effect of fs laser ablation on a transparent tissue. Histology images show that bubbles were generated inside the tissue without thermal damage, this method can be used to create a flap by connecting all these gas bubbles together. A flap on the cornea was created with 1.5 μJ pulse energy and 8μm spot separation. It was also demonstrated the position of gas bubbles in the epithelial cells can be changed by changing the laser focus (as shown in figure 2.22). The mechanism fs-LASIK surgery is illustrated in figure 2.23.
Figure 2.22. Histological sample of porcine cornea with fs optical breakdown. The subsurface ablation with change in laser focus towards the epithelial layer presented on the right side [2.105].

Figure 2.23. Different steps of laser assisted in-situ keratomileusis (LASIK) 1. Intrastromal subsurface cut performed on the cornea using spiral laser scan pattern 2. Another cut to prepare a lenticule, which depends on the refractive error of the eye (C shape with no ablation on one side). 3. Move the flap with no ablation on one side and extract the lenticule 4. Reposition the flap to change the refractive power of the eye [2.105].
In contrast to LASIK, USP lasers have also been successfully employed for corneal transplantation or keratoplasty. Researchers have shown that the use of USP lasers in keratoplasty can enhance the wound healing [2.106] and improve the astigmatism issue [2.107].

The application of USP lasers has also been investigated in neural surgery and was initially reported by Fischer et al. [2.108] and Loesel et al. [2.109]. It was found that negligible thermal damage occurred in the ablation of brain tissue (in the order of 1 µm). The design of a stereotactic hollow probe to deliver USP laser for neural surgery has also been demonstrated by [2.110]. Multiple tubes with a focusing lens and a deflecting mirror at the distal end of the tube were the main feature of the probe. However, such a system is not comparable to the optical fibres used in this work because of the non-flexible nature of the laser delivery system restricting its use for flexible endoscopic procedures. In 2016, Kerse et al, performed a rat brain tissue ablation using 800 fs pulse width laser with ultrafast burst of pulses [2.111]. There, it was demonstrated that an ablation rate of 2 mm³/minute in the rat brain tissue was possible with an average power of 2.7 W and no evidence of thermal damage.

In 2007, Henning et al. performed the first demonstration of vocal fold fs laser microsurgery [2.112]. A 780 nm wavelength and 130 fs laser pulse was used with 0.7 µJ pulse energy, 5 µm laser focused spot size and a pulse repetition rate (PRR) of 5 kHz. A galvoscanner was used to create 400x400 µm square pattern on the surface of the vocal fold tissue with 10% pulse overlap in the scan direction and laser line separation direction. The achieved maximum depth and thermal damage at the surgical zone was around 185 µm and ~1 µm respectively. The depth and shape were analysed using optical coherence tomography (OCT) imaging. The thermal damage was measured using histology images of the ablated region (Shown in figure 2.24). They also demonstrated the capability of fs laser to create sub-epithelial ablation without any damage to the adjacent tissue.

![Figure 2.24. The OCT image of the laser ablated porcine vocal fold and the corresponding histology image showing no thermal damage [2.112].](image-url)
Hoy et al. also demonstrated the subsurface ablation of porcine vocal fold [2.113]. The aim of this femtosecond laser resection was to create voids in the superficial lamina propria. The medical doctor has to inject a biomaterial to the void, this soft biomaterial is used to restore the viscoelasticity of the scarred vocal fold tissue to eliminate the voice disorders. The laser used for this operation had a pulse width of 150 fs, wavelength of 776 nm, PRR of 500 kHz and focused laser spot size of 1.47 µm. The size of the raster scanning pattern was 235 x 235 µm and was achieved by scanning the laser beam in the ‘x’ direction using a galvanometric scan head (75% pulse overlap) and moving the stage in the ‘y’ direction with a speed of 0.72 mm/s. Very small pulse energies were needed to start the ablation procedure in fs laser ablation. Pulse energies of 50, 100, 500 and 750 nJ pulse energies were applied, which corresponded to 3, 6, 30 and 45 J/cm². The 50 nJ pulse energy could create any ablation on the tissue surface but the 100 nJ created very small ablation, which was monitored using second harmonic imaging. The second harmonic generation of light is the basic principle of second harmonic imaging. A second harmonic microscope is capable to receive the contrast variations from the second harmonic light from the tissue as a result of ultrashort pulse laser irradiation. The 500 nJ and 750 nJ created subsurface voids in the tissue. Figure 2.25 shows the subsurface voids beneath the epithelial cell with a depth of 116 µm and minimal thermal damage. The same group developed a microsurgical probe based on hollow core photonic bandgap fibre and MEMS device [2.114] and in later works they used piezo electric actuators to scan the fibre for the microsurgery [2.115].

Figure 2.25. Second harmonic generation images of a subsurface void created in the porcine vocal fold (a) and the histology image of the ablated void (b) using 500 nJ pulse energy [2.113]
In 2016, Kaushik et. al. used a 100 µm thick porcine vocal fold tissue to investigate the ultrafast laser ablation characteristics [2.116]. A laser with a pulse width of 1.5 ps, PRR of 303 kHz and wavelength of 776 nm was used to ablate the tissue with a maximum fluence of 7.8 J/cm². The laser was transmitted through a kagome fibre optic probe, which was used to generate a lissajous pattern on the tissue surface at a frequency of approximately 1 kHz. However, no histology results are provided with this work to evaluate the thermally damaged region. A 1.5 ps laser pulse width has also been used by Lanvin et al. to demonstrate the subsurface ablation of atherosclerotic plaque beneath the endothelium of aorta in genetically engineered mice [2.117]. The laser ablated tissue areas were monitored by OCT imaging and histology analysis. The subsurface ablation was achieved with < 10 µm thermal damage. These studies give evidence to support the use of a picosecond laser surgery, in addition to the use of a fs laser. The laser was scanned inside the tissue using computer controlled XYZ stages. In this report different pulse energies were employed but the main ablation shown in figure 2.26 was performed with 4 µJ pulse energy and ~ 1 µm FWHM spot diameter. The applied pulse energy is one of the most important factors to determine the thermal damage in the tissue. It was concluded that for minimal surface damage in subsurface ablation, the laser should be focused beyond 15 µm inside the tissue with applied pulse energy <4 µJ.

![Figure 2.26](image)

Figure2.26. The subsurface ablation of an artery tissue, the ablated areas are marked within the ovals [2.117].

Later, Conkey et al. demonstrated the USP laser delivery through multicore fibre with laser pulse widths ranging from 500 fs to 2 ps [2.118]. It was observed a focusing efficiency (the percentage of laser power at the distal end of the fibre that reaches the
focal spot) changes due to non-linear effects in the fibre for all these pulse widths. The focusing efficiency change has been observed after 0.3 µJ input pulse energy due to self-phase modulation and pulse broadening in the output pulse (in 4600 core system). In a 10000 core system, the nonlinear effects were also observed in the fs regime. Material ablation was demonstrated using 2 µJ pulse energy, 1030 nm wavelength and 750 fs pulsed laser delivered via a multicore fibre of 10 cm long.

More recently, Kakkava et al. demonstrated the delivery of fs laser pulse through graded index (GRIN) multimode fibre for the ablation of the organ of corti in cochlea of mice [2,119]. Two different fibres of 200 µm and 400 µm core diameter were tested and the maximum focusing efficiency achieved was 28%. In the 200 µm core diameter fibre, the power coupling efficiency starts to decrease after 1 µJ pulse energy input to the fibre with pulse widths of 500 fs, 700 fs and 1 ps. In the 400 µm core diameter fibre, the power coupling efficiency has no serious change up to 10 µJ input pulse energy to the fibre (for 1 ps), but shows a slight decrease after 8 µJ pulse energy. On the other hand, for 500 and 750 fs laser delivery it was shown that a decrease in focusing efficiency occurs after 2 µJ of input pulse energy. The ablated tissue areas were analysed using two photon fluorescence imaging and bubbles with minimal damage were observed. The hollow core negative curvature fibre used in current work (explained in chapter 6) demonstrated more insensitivity to the non-linear optical effects compared to the GRIN fibre probe of 10 cm long. For 1.5 m of HC-NCF the power coupling efficiency changes after 12 µJ of input pulse energy (for 1 ps pulse transmission). However, the coupling efficiency change was observed after 7 µJ input pulse energy for 600 and 800 fs pulse widths. There should not be any non-linear effects observed in the HC-NCF for 2 ps pulse widths and longer. The focusing efficiency graph for the 200 and 400 µm core GRIN fibre is shown in figure 2.27.
The ablation of hard tissue using USP lasers showed the capability to create well defined craters with negligible heat affected zone and no micro cracks [2.120-121]. In 2004, Konorov et al. demonstrated the ablation of dental tissue using 40 ps pulses delivered through a hollow core photonic crystal fibre [2.122]. The 10 cm long fibre’s core was filled with gas to reduce the fibre non-linearities and to improve the optical breakdown threshold. However, from a practical point of view, the need for the fibre to be filled with gas presents significant technical challenges for use in an operating theatre. Schelle et al. performed investigations regarding the ablation rate on the dental tissue, ceramic composite and mammoth ivory using a picosecond laser of 8 ps pulse width, wavelength of 1064 and an average power of 10 W [2.123]. A 1mm² square cavity on the dental surface was made with 44% pulse overlap in the laser line separation direction and 84% pulse overlap in the scanning direction using a PRR of 500 kHz. The achieved ablation rate for this application is shown in figure 2.28. The maximum ablation rate of dentin and enamel were 7.69 and 6.41 mm³/minute respectively.
The preparation of dentin and enamel using a 400 fs laser has been performed by Chen et al. [2.124]. A 24 µm diameter spot was used to create different pulse overlap and laser fluences. The relation of these parameters including pulse energy have been analysed along with the laser ablation efficiency. The depth analysis of the ablated zone was carried out using a 3D microscope. The laser pulse was scanned by galvanometer scan head and it was concluded that a fine ablation can be achieved by using a moderate laser fluence (4.69 J/cm²) with the pulse overlap adjustment and an optimized number of ablation patterns (shown in figure 2.29).

Figure 2.28. The ablation rate versus fluence of different materials, dentin and enamel [2.123]

Figure 2.29. The ablation of dental tissue using 4.69 J/cm² with scanning line spacing of 12 µm a and c) enamel with number of scanning layers 25 and 200 respectively. b and d) dentin with number of scanning layers 25 and 200 respectively [2.124].
The application of USP lasers in bone tissue surgery has been getting more attention because of its capability to create thermal/mechanical damage free craters [2.125-126]. 1µm/pulse ablation rates have been achieved in different research works, which was using a peak fluence of 2 J/cm² and a PRR of 10 kHz [2.127-128]. Different studies have been performed on porcine cortical bone using USP lasers [2.129-130]. In 2013, An et al. performed a feasibility study on porcine cortical bone using a laser with a pulse width of 170 fs, wavelength of 800 nm, focused spot diameter of ~30 µm and a fluence of 19.3 J/cm² [2.130]. Ablation using a concentric circles scan pattern and spiral helical pattern were tested. The focus was adjusted when ablating multiple patterns. A crack free and thermal damage free smooth surface in the ablated crater was achieved.

Using small pulse energies (<5 µJ), the subsurface ablation using femtosecond laser was effective means to create a surgical effect inside the tissue because of the high peak power density and laser fluence achieved at the beam waist was sufficient to generate optical breakdown. On the other hand, picosecond lasers with small pulse energy and similar spot sizes are not able to generate equivalent peak power densities created by fs lasers for plasma formation or secondary effects. To achieve the same peak power density, the ps laser needs to increase the pulse energy. To achieve the same laser fluence, the ps laser needs to increase the pulse energy or to decrease the focused laser spot size. Some research has been conducted where the spot size is < 5 µm [2.113, 116-117], to reduce the spot size again is optically a complex process and it also would increase the surgery time due to the small interaction volume. The easiest method is to increase the pulse energy. However, the increase of pulse energy can cause thermal damage to the tissue. Therefore femtosecond lasers with low pulse energy and small spot size are favoured for subsurface USP laser surgery. Self-focusing is also an important effect that limits the maximum ablation depth and precision in bulk tissue femtosecond laser surgery [2.131].

The peak power of the laser pulse depends on the self-focusing effect. When the peak power exceeds a critical power [2.131] the self-focusing effect occurs and the pulse will collapse and be focused before the original intended focus. Miclea et al. found that the critical power for femtosecond laser ablation in porcine cornea was 1.2 MW [2.132]. The self-focusing effect can be avoided by using a smaller spot size with the capability to achieve the plasma generation [2.131]. However, using low pulse energy and a very small focused laser spot size (<5 micron) can significantly reduce the rate and efficiency of ablation. The picosecond laser (in the ultrashort pulse regime) is an ideal choice to avoid the self-focusing effect and to resect larger tissue areas with a few tens of diameter of spot
size. The optimal scanning strategy, laser spot size, applied pulse energy and fluence can greatly improve the ablation rate and thermal damage during tissue resection.

2.4. Relevant animal models for developing picosecond laser resection procedures

According to Cancer Research UK data, the second highest number of cancer deaths in the United Kingdom occurs due to bowel cancer [2.133]. The early diagnosis and treatment of the colon cancer precursor, adenomatous polyps, are important to reduce the rate of mortality. The stages of colon cancer are determined by the extent of spread through the layers of the bowel wall and whether the localized tumour has spread to lymph nodes or distant organs. The surgical removal of colon polyps or cancers based on conventional techniques may lead to two of the most important post-surgery complications, bleeding and perforation [2.134]. In this work the relevance of ultrafast laser in colon tissue resection for minimal perforation or thermal damage is demonstrated. In order to develop the process pre-clinically a reliable and relevant animal model had to be used. Prior work has established pig tissue as an efficient medical model for several studies related to human diseases [2.135]. The similarity in shape and morphology of the pig colon and human colon and the availability of tissue from the animal research institute at the Roslin institute Edinburgh are the main reasons to select pig colon for our studies. The sheep lung tissue collected from Moredun animal research institute Edinburgh and the mice colon tissue are provided by Leeds institute of medical research at University of Leeds. The main challenge faced during the study was the availability of tissue at the right time, which is constrained by a protocol for performing the biological tissue experiments approved by Heriot Watt University. Figure 2.30 shows a schematic of the colon structure. The innermost layer of the colon is known as the mucosa, which consists of epithelial cells arranged in crypts, connective tissues and a thin smooth muscle layer at the base. The submucosa is composed of connective tissue with blood and lymphatic vessels. The muscularis propria is the main muscle layer of the bowel and has an inner circular and an outer longitudinal layer. The outermost adventitia or subserosa is composed of connective tissue with the larger blood and lymphatic vessels along with lymph nodes, clearly shown in figure 2.31. The objective is to develop a precise way to resect colonic polyps or cancer cells in the mucosa and submucosa, i.e. the area in between the two red circles in figure 2.30. All the tissue collection, handling, preparation and fixing methodologies used in this work were developed by Dr Rainer Beck in collaboration with the clinical partners and animal research institutes.
The pig colon tissue has been collected locally from the Roslin research institute Edinburgh. The sheep lung and mice colon tissue have been collected from Moredun animal research institute Edinburgh and medical research wing of University of Leeds respectively. The figure 2.32 represents the histology image of healthy human colon, mice
colon and laser ablated sheep lung tissue. The colon tissue of pig and human are similar in structure and composition. The mice colon is very small and delicate in nature. The sheep lung is composed of thin walled alveoli surrounded by squamous epithelium. A pilot study has been performed on healthy and cancerous lung tissue using 6 ps and 515 nm wavelength laser. The main animal model used in this study was porcine model. The ovine and murine model are used for a few experiments to prove the feasibility of picosecond precision laser surgery in other animal models and organs.

Figure 2.32. Histology images of a) human colon [2.136] b) mice colon c) sheep lung. 1, 2 and 3 represents mucosa, submucosa and muscularis propria respectively.

2.5. Possibility of theoretical modelling in plasma induced colon tissue ablation

The theoretical modelling of plasma mediated and pulsed laser ablation has been carried out by different research groups on metals [2.137], hard tissues [2.125, 138] and soft tissues [2.139]. In metals the modelling is easier because the property of the material homogenous and the melting thresholds and heating estimate have been theoretically predicted using two temperature model in metals [2.140-141]. The two temperature model is a theoretical model used to predict the dynamics of metal after the ultrashort pulse laser interaction. The limitation of the two temperature model is explained in [2.142]. Electron heat capacity, electron heat conduction, electron relaxation time and reflection in tissue surface is very difficult to find out when using two temperature model for laser tissue interaction modelling. In hard tissue the modelling is not as difficult as
that for transparent and turbid media. Most of the USP laser ablation studies are conducted in water. Researchers have used the properties of water for the theoretical prediction and validation of laser ablation in soft tissue [2.143-144]. Recently, the plasma mediated ablation of porcine sclera using a 2D finite element model has been performed [2.143]. Differences in the theoretical and experimental work were observed because of the number of assumptions used. The assumptions are, 1) Radiative and convective heat losses were not considered for the simulation 2) size of the simulated sample was much smaller than the actual sample 3) Temperature increase in the sample below the plasma threshold regime is neglected for the heat analysis model. They found discrepancy in experimental and theoretical data because of this assumption. The theoretical modelling of thermal interaction associated with ultrashort pulsed laser ablation has been performed in skin tissue model by Jiao et al. in 2011 [2.145].

The absorption coefficient of the target sample and the plasma are important parameters for the plasma mediated ablation and heat conduction modelling. In the colon the mucosa, submucosa and muscularis propria have different optical and mechanical properties [2.146-148], which may be the main issue in considering colon tissue as a model for theoretical predictions of USP laser ablation. The mucosa is composed of epithelium, lamina propria and muscularis mucosa. The epithelium consists of lower columnar to cuboidal cells, crypts (open to surface epithelium or surrounded by a fibroblast sheath), goblet cells. The lamina propria contains the capillaries and muscularis mucosa is a smooth muscle layer [2.149]. The second part of the submucosa is composed of loose connective tissue and blood vessels. The muscularis propria is the third layer, which has the strong mechanical strength and resistance to the laser ablation (which was evident in our experimental work). To develop a reliable colon tissue laser ablation model, considering all these layers into one layer may not be appropriate. It was therefore decided that developing a reliable model for this laser ablation of colonic tissue was outside the scope of this work which focuses on experimental determination of optimal laser parameters validated using profilometry and histological analysis.

2.6. Evolution of Hollow Core Negative Curvature Fibre: Technology and Medical Applications

The medical application of optical fibres started in the early 1950s when fibre optic bundles were used to image inside the body [2.150]. The flexibility of fibre optic cables makes them an ideal choice for endoscopes used in minimally invasive surgery. One of
the main advantages of optical fibre is that as the most common optical fibres are made from silica they are robust and chemically inert and present no negative side effects when in contact with biological tissue. The invention of the laser and communication optical fibres triggered a growth in the research into fibre optic delivery of laser pulses and application to the medical field. Initially, research focussed on standard multimode and single mode solid core silica fibres for different medical applications such as medical imaging [2.151-153], laser surgery [2.154-155] and laser therapy [2.156-157]. In such standard solid core step index fibres the light guidance is based on total internal reflection (TIR) established with a refractive index difference between the core and cladding.

Recently, Photonic Crystal Fibres (PCF) have been developed which also guide due to a refractive index difference between the core and the cladding. In the case of the PCF a silica core is surrounded by hexagonal spaced air-silica photonic crystals with a lower effective index than the core [2.158]. In 2001, Hartl et al. used such PCF in a medical application to generate high resolution optical coherence tomography (OCT) images of a Syrian hamster’s cheek pouch [2.159]. The highly nonlinear properties of the PCF was used to generate a supercontinuum laser, which was used as an input to the OCT device. In another example, a multimodal nonlinear optical microscope dependent on the nonlinear property of the PCF was used to image the arterial tissue in an ex vivo rabbit model [2.160]. However, this type of PCF has not been used extensively in medical applications because of its high nonlinearity, low damage threshold and high birefringence.

The concept of the photonic bandgap was first introduced by Yablonovitch [2.161] and John [2.162]. In 1995, Birks et al. proved the possibility of a photonic bandgap fibre that guides light by Bragg reflection instead of TIR [2.163]. Later, Cregan et al. practically demonstrated the light guidance in Hollow Core Photonic Bandgap Fibre (HC-PBF) [2.164]. The HC-PBF showed superior quality in transmission loss, nonlinearity and damage threshold when compared to the PCF. However, the main disadvantage of the PBF is reduced transmission bandwidth in comparison with other fibres. In 2002, a new type of hollow core fibre was developed by Benabid et al. with higher transmission bandwidth [2.165]. This fibre is referred to as a ‘Kagome’ fibre because of the kagome like structure of the lattice in the cladding shown in figure 2.33c. The potential of HC-PBF for ultrafast and high peak power pulse delivery made them suitable candidates for a range of medical applications including surgery. For example in 2016, femtosecond laser pulse delivery through kagome fibre was applied for microsurgery on porcine vocal
folds [2.116]. A frequency doubled erbium doped fibre laser at 776 nm and 1.5 ps pulse width was used for the microsurgery [2.166]. The full power of the laser is focused to the fresh porcine vocal folds with a spot diameter of 4.38 µm and 7.8 J/cm² fluence. They performed the laser ablation on the tissue surface with 5 mm/s fibre scanning speed and achieved a 40 µm width and 43 µm deep crater.

The importance of the core wall shape in the light guidance property of the Kagome fibre was also reported [2.167] and Pyramikov et al. fabricated the first negative curvature fibre in 2011 [2.168] and shown in figure 2.33 b. The figure 2.33 a and b show the positive and negative curvature cores respectively. In the case of positive curvature speciality fibre the cladding part of figure 2.33a may consist of periodic structures. In figure 2.33a, the core wall is circular in nature, which is considered as positive curvature of the fibre core. The negatively curved core wall is shown in figure 2.33b. This fibre exhibits low attenuation because of the negative curvature core boundary. In 2012, F. Yu et al. fabricated a Hollow Core Negative Curvature Fibre (HC-NCF) with “ice-cream cone” shaped cladding capillaries (see figure 2.34d) which was developed for the transmission of 3-4 µm wavelength of light [2.79].

![Figure 2.33. Fibre core with positive (a) and negative curvature (b) [2.170].](image)

The hollow core means that there is low overlap of light with the glass (silica) and hence the fibre transmits light with low loss in a wavelength region where bulk silica has high attenuation [2.169]. During the same year Urich et al. demonstrated the capability of HC-NCF delivered laser pulses in surgery. An Er:YAG laser with a wavelength of 2.94 µm was transmitted through the fibre with a coupling efficiency of 35%. However, they successfully demonstrated the laser ablation on a porcine bone [2.78] with the fibre delivered pulses. This again demonstrates the advantage of the hollow core fibre geometry which enables silica based fibres to transmit in region of high bulk attenuation. In 2013 Jaworski et al. successfully demonstrated high peak power ultrafast laser delivery through
8 cell HC-NCF for micromachining applications [2.170-171] although a disadvantage of this fibre design was the multimode transmission of laser light in 1 μm regime. Figure 2.34 a, b, c and d shows the evolution HC-NCF of In 2015, Hazel et al. demonstrated the capacity of HC-NCF delivered femtosecond pulses for the enhancement of drug delivery in the mammalian skin [2.172]. The touching points in the cladding structure act as additional waveguide and increase the transmission loss of the fibre. To reduce the loss of HC-NCF Kolydin et al proposed an open boundary core wall geometry HC-NCF in 2013 [2.173] shown in figure 2.35.

Figure 2.34. Scanning electron micrograph images of different speciality fibres. a) Endlessly single mode solid core photonic crystal fibre [2.158] b) Hollow core photonic bandgap fiber [2.164] c) Hollow core photonic crystal fibre with kagome lattice cladding [2.165] d) 8 cell (ice cream cone shape) Hollow core negative curvature fibre [2.79].

Figure 2.35. Open boundary core wall structure of HC-NCF [2.175]
The conventional surgical methods and ultrashort pulse laser tissue resection modalities are explained in this chapter. The underlaying mechanism of plasma mediated ablation and its secondary effects provide an overview regarding the ultrashort pulse laser surgery. The evolution of HC-NCF is also explained in detail to understand the microstuctured optical fibre family. The ultrashort pulse picosecond laser ablation in porcine colon tissue is presented in next chapter.

2.7. References


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III, European Conferences on Biomedical Optics, Munich, Germany, 2007, SPIE, 6632.


[2.136] Histology Guide: Large Intestine,


Chapter 3

Ultrashort pulse picosecond NIR laser resection of colon tissue in a porcine model

3.1. Introduction

Energy absorption and heat diffusion are the most important linear processes regulating long pulsed laser surgery procedures [3.1], which is prone to thermal damage to the adjacent healthy tissues in the surgical zone. The introduction of ultrashort pulsed lasers into the surgical application potentially minimizes further the thermal damage and improves the surgery time when compared to long pulsed laser and other conventional surgical techniques. The surgical procedure in Ophthalmology, Neurosurgery, Cancer surgery and Polypectomy requires high precision and minimal heat affected zone. The goal of this study is to investigate the efficacy of an ultrafast picosecond laser in colon surgery with high precision and minimal thermal necrosis.

Over the last few decades, picosecond (ps) and femtosecond (fs) lasers have been deployed in laser surgery research related to soft [3.2-5] and hard tissue [3.6-8] ablation. These lasers have the ability to offer precise and rapid resection with minimal thermal and mechanical damage to the adjacent tissue in the surgical zone [3.9]. The NIR lasers were used for surgical procedures in ophthalmology, neurosurgery and dentistry [3.10-14]. The goal of these surgeries are to create negligible thermal damage to the adjacent tissues. The introduction of 1030 nm picosecond pulses and different laser scanning techniques into the surgical procedure of endoluminal gastroenterology is the goal of this work. The pre-clinical evaluation of the 1030 nm picosecond pulse in colon surgery is presented in this chapter. Additionally, hollow core negative curvature fibres developed by University of Bath are designed for the flexible delivery of 1030 nm ultrashort pulses, which is another important factor to select the 1030 nm for the evaluation of its surgical capability.

Plasma mediated tissue ablation is a non-thermal resection modality with enhanced precision compared to other conventional surgical modalities. Currently surgeons are using electrocautery devices to resect colon tissue, which lack accuracy and are prone to bowel perforation. The enhanced precision of high peak power lasers in colon surgery could make them an indispensable tool as an alternative to current surgical methods. The results of experiments on laser resection of porcine colon by means of ultrafast laser are analysed and presented in this chapter.
3.1.1. Key laser parameters associated with tissue ablation

**Wavelength:** The wavelength of the laser light is one of the key parameters associated with long pulsed laser ablation. In long pulsed laser ablation, each wavelength will have unique absorption characteristic with the tissue chromophores. However, in ultrashort pulse laser ablation, plasma mediated ablation is independent of wavelength because of the nonlinear absorption process involved in plasma formation in the tissue.

**Pulse width:** Pulse width is another important parameter for ultrashort pulse laser ablation. The time duration of a pulse determines the pulse width of a laser. The pulse width in the ultrashort pulse regime (<10 ps) is suitable for tissue resection because of its capability to generate high peak power density (>10\(^{11}\) W/cm\(^2\)) at the tissue surface using relatively low average powers.

**Repetition rate:** Pulse repetition rate of a laser defined is the number of pulses per second. High pulse repetition rate (> 1 kHz) with high pulse overlap has the capability to generate secondary effects of plasma formation such as cavitation and shock wave. Optimization of pulse repetition rate and pulse overlap is paramount in ultrashort pulse laser ablation. The pulse energy is the ratio of average power and pulse repetition rate.

**Peak power:** Peak power of the laser is an important parameter in ultrashort pulse laser resection. Peak power is the ratio of pulse energy to pulse width. The peak power density determines the formation of plasma on the tissue surface and it can be calculated by using peak power and focused spot size. In ultrashort pulse laser resection, if the peak power exceeds 10\(^{11}\) W/cm\(^2\) then plasma will generate on the tissue surface.

**Rayleigh length:** The Rayleigh length of the laser is a key parameter in laser material processing or tissue ablation. The Rayleigh length is defined as the distance from the smallest beam radius (beam waist) where the beam radius is \(\sqrt{2}\) times bigger. The porcine tissue surface has an average roughness of 30 µm. The lasers employed in the experimental work in this thesis have a large Rayleigh length compared to the roughness of the tissue surface. The Trumpf laser has a Rayleigh length range of 732 µm (1030 nm) and 441 µm (515 nm) when using a galvanometer with an F-theta lens of 160 mm focal length. The Carbide laser has a Rayleigh length range of 235 µm (1030 nm) when using galvanometer with an F-theta lens of 100 mm focal length. For fibre delivered beam, the Rayleigh length is 124 µm (1030 nm) with a focusing lens of focal length 20 mm. All of these Rayleigh lengths were greater than the measured tissue roughness. The effect of
tissue roughness in tissue ablation is negligible when a laser with a large Rayleigh length is used for ablation.

**Pulse overlap:** Laser pulse overlap is a factor that must be considered in order to avoid undesirable heat accumulation effects during tissue ablation with picosecond lasers. Optimized pulse energy and pulse overlap can generate a clean ablation with negligible thermal damage, which was investigated in this thesis.

### 3.2. Experimental Setup

#### 3.2.1. Trumpf TruMicro 5x50 laser for tissue resection

![Schematic of the laser system for tissue resection.](image)

The configuration of the experimental system based on direct laser ablation through a galvanometer scan head is shown in figure 3.1 using a Trumpf TruMicro 5x50 laser. A collimated laser beam with a centre wavelength of 1030 nm, pulse width of 6 ps, maximum output power of 44.5 W and pulse energy of 111 µJ was used for the tissue resection experiments. The maximum pulse repetition rate (PRR) of this laser is 400 kHz with a beam quality ($M^2$) value of <1.3. A galvanometer scanhead is deployed to focus the laser beam to the inner lining of the colon tissue for a definite resection. A suitable F-theta lens of focal length 160 mm is employed to focus the near infrared (NIR) laser to the tissue surface. The mirrors attached to the high speed galvanometer motors are used to scan the laser light in x and y directions. The figure 3.2 shows how the laser spots are scanned across a 1 mm$^2$ area. The laser beam is raster scanned only in one direction and laser beam is off during the fly back time, shown in figure 3.3 as dotted lines. The speed of the motor can be varied, (marking speed) which dictates total resection time. The
measured Rayleigh length of this laser was 732 µm. The Rayleigh length has been measured using the scanning slit beam profilometer and is defined as the length where the beam mode area becomes double in size compared to the lowest beam mode area. Table 3.1. shows the parameters related to Trumpf TruMicro 5x50 laser.

Figure 3.2. The laser pulse overlap pattern in the scanning direction and line separation direction

Figure 3.3. The laser scanning pattern showing the flyback line in dotted arrows
Table 3.1. Parameters of Trumpf TruMicro 5x50 laser (1030 nm)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
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</tr>
<tr>
<td>Pulse Repetition Rate</td>
<td>1 to 400 kHz</td>
</tr>
<tr>
<td>Pulse Energy</td>
<td>Up to 111 µJ</td>
</tr>
<tr>
<td>Pulse Width</td>
<td>6 ps</td>
</tr>
</tbody>
</table>

3.2.2. Light Conversion Carbide laser system for tissue resection

An industrial laser (Light Conversion CARBIDE) with a central wavelength of 1028±5 nm, a range of base repetition rates from 60 kHz to 1 MHz and a tunable pulse width ranging from 232 fs to 10 ps was used. A maximum average power of 5 W and a base repetition rate of 60 kHz was used for the application. This system uses a scan head with F-theta lens of focal length 100 mm (shown in figure 3.4), and Figure 3.4a shows the spiral scan pattern that is employed in this case, a detailed analysis of spiral scan pattern is presented in section 3.2.5.1. Table 3.1. shows the parameters related to Carbide laser system.

Figure 3.4. Schematic of the laser system for tissue resection. a) Schematic of the spiral scan pattern at the colon tissue surface during picosecond laser ablation
### Parameters of Carbide laser (1030 nm)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>1030 nm</td>
</tr>
<tr>
<td>Pulse Repetition Rate</td>
<td>60 kHz to 1 MHz</td>
</tr>
<tr>
<td>Pulse Energy</td>
<td>Up to 83 µJ</td>
</tr>
<tr>
<td>Pulse Width</td>
<td>232 to 10 ps</td>
</tr>
</tbody>
</table>

#### 3.2.3. Determination of focal spot size

The focused spot size of both the Trumpf and Light Conversion laser systems was measured using a scanning slit beam profiler (DataRay, Beam Map 2). The important part of this device is a rotating disc with four slit pairs at four different positions parallel to the z axis plane shown in figure 3.5. The slits are placed at $z=0$ (reference plane), $d$, $-d$ and $+4d$, the value of $d$ is 500 µm. The real time measurement of the $M^2$ value of a laser beam is also possible with this device. The waist size ($1/e^2$) of the focused beam was measured as approximately 34.4 µm in diameter (shown in figure 3.6 and 3.7) with a beam quality ($M^2$) of 1.3 for Trumpf laser system. The two and three dimensional intensity distribution of 1030 nm laser focus is presented in figure 3.6.

Figure 3.5. Schematic of the slit configuration of a scanning slit beam profiler [3.15]
Figure 3.6. Two dimensional (a) and three dimensional (b) isometric plots of a picosecond 1030 nm laser beam at the colon tissue surface

Figure 3.7. Horizontal and vertical cross section of the beam waist diameter at focus

In ultrashort pulse laser surgery, the aspect ratio of the laser resected tissue zone is mainly confined by the spot size of the laser. The use of small spot size leads to higher laser irradiance, which can trigger an efficient surgical effect in targeted tissue. Hence, the selection of laser spot size in USP laser surgery is extremely important.

Figure 3.8 shows the beam waist diameter from the laser focus to 1.1 mm below the laser focus. The \((1/e^2)\) beam diameter at the laser focus was 18 \(\mu\text{m}\) for carbide laser system. It has been observed that the horizontal and vertical diameter of the laser pulse was same up to 300 \(\mu\text{m}\) below the laser focus, where the beam diameter is double in size compared to beam diameter at focus.
Figure 3.8. Horizontal (X) and vertical (Y) diameter of the 1030 nm laser pulse from the laser focal point

3.2.4. Preparation and positioning of tissue sample

A protocol had to be devised that allowed laser resection trials on clinically relevant material. In order to achieve meaningful results that would be comparable to live tissue, the samples needed to be kept in a fresh state until the laser processing trial had been completed. Then immediately after the laser trials, the samples could be fixed to preserve the cellular structure for histological analysis. The pig colon specimens were harvested from a mature pig (euthanised under a Schedule 1 killing), obtained from Roslin Research Institute, Edinburgh UK within one hour of post-mortem. Laser processing needed to be carried out no longer than 48 hrs after harvesting the tissue from the euthanised pig. It has been preserved in phosphate-buffered saline (PBS) on ice to maintain the cellular integrity and tissue morphology, which was the advice from our clinical collaborators. During this initial period, after death, tissue degradation is a relatively slow process [3.16] therefore allowing time to perform tests whilst it is still clinically relevant. The dissected colon tissue samples were kept in a phosphate-buffered saline (PBS) buffer solution until the laser resection. The tissue sample was placed on a DispoCut (Cell Path Inc.) board which has the capability to resist moisture and is helpful for an accurate dissection using a surgical blade. The next procedure was to place and secure the tissue sample in a petri dish, which is then placed on a computer controlled XYZ translational stage for laser processing. All experimental procedures were conducted according to the protocols approved by the Heriot Watt ethics committee on biological tissue handling. Additionally,
the appropriate health and safety measures have been implemented which the University biological safety officer oversees. After laser resection, one set of colon tissue samples are placed in a cellstor pot filled with 4% aqueous solution of formaldehyde. An example picture of the porcine colon after laser resection is shown in figure 3.9a. These samples are used for the histological analysis of the laser resected part in order to measure the dimensions of the laser excision and width of coagulated necrosis. The other set of samples are stored in cellstor pot containing 3% aqueous solution of glutaraldehyde which are used for 3D profilometric studies using the Alicona system after glutaraldehyde fixation (shown in figure 3.9b).

The Aerotech (for Trumpf laser system) and Newport (for Carbide laser system) XYZ stages were the integral part used to position the tissue sample at the focus of the laser beam. A CCD camera with 20X objective attached to a lens tube was used to find the laser focus for tissue resection (±10 µm accuracy). The procedure first involved finding the accurate laser focus under the galvanometer by machining lines on a flat metal sample with different z positions and recording the x, y and z coordinate value of the accurate focus position. This laser marked sample is then moved to the focus of the microscopic objective and the corresponding x, y and z coordinate values of this second position is noted. The offset in these two set of values is used to align the tissue with the focus of the laser. For the tissue resection experiments, the fresh tissue sample is positioned under the microscope and brought into focus. The tissue sample is then translated from the microscope focus (using the pre-determined offset) to under the galvanometer so it will then be positioned at the laser beam focus.

Figure 3.9. Porcine colon after a) laser processing and b) after glutaraldehyde fixation. The black ink is used to identify the end markers for the pathologist.
3.2.5. Laser parameters for tissue resection

3.2.5.1. Pulse overlap strategy

The pulse overlap strategy is based on either a raster scan pattern or an Archimedean spiral pattern. A high laser pulse overlap was used for the initial trials of tissue resection. Figure 3.10 shows the schematic representation of a pulse separation in a raster scan pattern, which was designed with the help of TruTops software. The pulse separation or spacing distance in both laser scanning and laser line direction determines the percentage of pulse overlap in both directions. For the Trumpf TruMicro system the focused laser spot diameter was measured as 34.4 µm (see section 3.2.3) and a pulse separation distance of 3.4 µm has been used to achieve an approximately 90% pulse overlap. The laser scanning speed is varied according to the pulse repetition rate (PRR) to maintain this desired pulse overlap percentage. The pulse overlap percentage can be determined by the equation (3.1),

\[
\text{Pulse Overlap Percentage} = \left(\frac{\text{Focused Spot diameter} - \text{Pulse separation distance}}{\text{Focused spot diameter}}\right) \times 100 \quad (3.1)
\]

The pulse overlap percentage in the laser scanning direction of higher pulse repetition rate can be regulated by changing the beam scanning speed of the galvo scanhead. Different values of pulse overlap percentage in the laser scanning direction and laser line separation direction were used for the tissue resection.
The repetition rate has an important role to control the total time in the laser surgery because in higher repetition rate operation the time interval between consecutive pulses are very low which influences the total resection time. However, it has been observed that a high amount of thermal damage can occur after high repetition rate operation of laser with high pulse overlap, (which is discussed in section 3.4.3). To achieve a perfect surgical effect, the thermal or mechanical damage to surgical zone due to heat accumulation, shockwaves and cavitation effects should be minimum or negligible.

In different repetition rate experiments with the same pulse overlap percentage, the total energy applied to the tissue surface was same but the time of operation was different according to the repetition rate. The total energy deployed on a 1 mm$^2$ raster scan pattern can be calculated by equation 3.2,

$$\text{Total energy} = \text{Total number of lines} \times \text{Number of pulses in a line} \times \text{pulse energy} \quad (3.2)$$

The total number of lines in a 1 mm$^2$ pattern with 3.4 µm separation is 294. The total number of pulses in one line also 294 (90% pulse overlap). Therefore for a pulse energy of 107 µJ (which was the maximum pulse energy used in the experiments),

$$\text{Total energy} = 294 \times 294 \times 107 \, \mu\text{J} = 9248652 \, \mu\text{J} = 9.2486 \, \text{J}$$

The time taken to complete the raster scan pattern in different PRR operations are calculated using a formula [3.17],

$$t = \frac{l \times n \times N}{\gamma} \quad (3.3)$$

Where $n$ is the number of scanning lines within one ablation layer, $l$ is the length of the pattern, $N$ is the number of ablation layers, and $\gamma$ is the laser scanning speed. The flyback time of 294 lines also added to the above equation for an accurate calculation, which has a typical speed of 2000 mm/s. 86.61, 8.78 and 4.46 s were the time taken to complete one iteration of 1 kHz, 10 kHz and 20 kHz pulse repetition rate operation respectively. The rate of energy delivered to the tissue surface in 1 mm$^2$ raster scan pattern was 0.106, 1.05 and 2.07 J/s for 1, 10 and 20 kHz PRR operation respectively for 107 µJ pulse energy.

It has been noted that a high amount of energy is interacting with the tissue in one second operation of 10 and 20 kHz PRR compared to 1 kHz PRR operation. The time duration of the surgery is an important factor determining the performance of ultrafast laser surgery techniques.
The spiral patterns were also used to ablate the tissue, a schematic is shown in figure 3.11. Figure 3.11a shows that a pattern with zero percent overlap in the laser scanning direction and line separation direction. Figure 3.11b represents a schematic of spiral scan line pattern used for the ablation studies, which was designed with the help of Laser Desk software. The length of the spiral can be calculated using equation 3.4 [3.18]. The answer is validated and the analysis of calculation method is presented in [3.19-20].

\[ L = \left( \frac{\pi R (D + d)}{D} \right) / 2 \]  

\[ (3.4) \]

\( L \) is the length of the spiral, \( R \) is the number of spiral turns, \( D \) is the total diameter of the pattern and \( d \) is the inner diameter of the pattern, which is marked in figure 3.11b. The number of spirals in the pattern is the ratio of the radius of the pattern \( (D/2 - d/2) \) (excluding inner radius \( d/2 \)) to the laser line separation distance (which determines the pulse overlap percentage in laser line separation direction).

Consider a spiral scanning pattern with 2 mm diameter (D), inner diameter 40 µm (d) with 0% overlap pattern in scanning direction and 90% (1.8 µm separation for an 18 µm spot diameter) overlap in line separation direction. The number of turns and total length will be 554 and 1743 mm respectively. The total time taken for completing one pattern is the ratio of the total length of the spiral to laser scanning speed.
3.2.5.2. Laser pulse energy and laser fluence

The laser ablation on porcine colon tissue was carried out on a number of samples. On each sample five separate areas were ablated with the same scanning pattern but different laser pulse energies. The typical area scanned was 1 mm by 1 mm. The pulse repetition rates investigated initially were 1 kHz, 10 kHz and 20 kHz. The average laser fluence of a picosecond laser was calculated from the following formula [3.21],

\[ F = \frac{E_p}{\pi \omega_0^2} \] (3.5)

Where \( E_p \) is the picosecond laser pulse energy and \( \omega_0 \) is the radius of the laser spot at the focus (for the Trumpf laser this 17.2 microns and for the Carbide laser this was 9 microns).

The laser pulse energies (Trumpf laser) investigated were 107, 88.5, 70.5, 52.5, 34.5 µJ and the corresponding average fluence was approximately 12, 10, 8, 6 and 4 J/cm\(^2\). The aim of using different fluence was to monitor the changes in ablation when applying a maximum (available) to minimum laser fluence on the tissue surface. These experiments were mainly focused on assessing the ablation depth and thermal damage according to the change in laser pulse energy and average laser fluence.

For the majority of the experiments the pulse overlap was maintained at 90%. The analysis of high pulse overlap interaction with tissue was the initial aim for selecting 90% pulse overlap. However, some trials (see section 3.4.3) were carried out to assess the effect of pulse overlap. In these experiments the pulse overlap was varied from 94% to 88%, using a pulse energy of 88.5 µJ, in order to monitor the changes in the ablation depth and thermal damage. As with the 90% pulse overlap experiments the pulse overlap percentage in the laser scanning and the laser line direction was kept the same.

18 and 13 J/cm\(^2\) laser fluences (Carbide laser) were used to ablate the tissue using spiral scan pattern of 2 mm diameter. For different experiments, different pulse overlap strategies were used in the laser scanning direction. The pulse overlap percentage at the laser line separation direction is kept constant (90%) to see the ablation changes with different pulse overlap percentage (90, 70, 50, 20, 0) in the scanning direction.
3.3. Three dimensional (3D) profilometric and histology analysis of tissue

An Alicona Infinite Focus (IF) profilometer (shown in figure 3.12 b) is used to analyze the laser ablation profile of tissue specimens providing high resolution analysis via an optical 3D micro co-ordinate measurement system. A 5x objective (max. lateral & vertical resolutions of 4.38 and 0.428 µm respectively) is applied to analyze the laser ablated, glutaraldehyde fixed colon tissue samples with lateral and vertical resolutions of 5 µm and 2 µm respectively. A focus-variation technique is utilized in the Alicona IF device, which can characterize the depth and surface profile information of laser ablated parts of the tissue. The Alicona IF microscope captures a number of 2D images between the highest and lowest focal plane of the laser ablated zone of a tissue sample. The processing of these 2D images leads to the accurate reconstruction of the tissue surface profile in the form of a 3D image.

![Figure 3.12](image.png)

**Figure 3.12.** Three-dimensional surface profile of the laser ablated zone (a) image of the 3D optical profilometer (b). Horizontal (c) and vertical (d) depth profile of the same crater.

In Figure 3.12, 3.12a shows the 3D profile of the laser ablated zone created using the galvanometer system with 7 J/cm² laser fluence, 90% pulse overlap in the 1 mm² raster scan pattern. Figure 3.12c and 3.12d shows the horizontal and vertical cross sections of the surgical zone and the depth profile measured through the centre of the cavity. The lines c1 and d1 in figure 3.12a through the centre of the crater represent the depth profiles in figure 3.12c and 3.12d respectively. The average depth and standard deviation of the
Ablated zone was calculated by taking the mean value of the individual average depth and standard deviation of horizontal and vertical depth profiles.

For the histology analysis, the samples were fixed in formalin and sent to the pathology lab (at the University of Leeds). Fixed tissues were processed on a Leica ASP200 tissue processor (Leica UK) on a routine overnight programme. After embedding in Cellwax (Cell path UK), 4 micron sections were cut on a Leica 2235 microtome and placed on glass microscope slide before staining with Haematoxylin and Eosin. The process of Haematoxylin and Eosin (H&E) staining on the tissue is used to understand the resection depth and thermal injury to the surgical zone during the laser resection.

### 3.4. Porcine Tissue resection using Trumpf Laser

#### 3.4.1. Tissue resection using out-of-focus laser pulses

An experiment of tissue resection using out-of-focus pulses was conducted to investigate the effect of focal position in relation to the tissue surface. The ultrashort pulses with approximately 90% pulse overlap, 1 kHz pulse repetition rate and 88.5 µJ pulse energy were used to perform 1mm$^2$ raster scan patterns on the tissue surface. The measured Rayleigh length of the laser beam under F-theta lens (160 mm focal length) was 732 µm. The size of the increment change in focal position was fixed at 400 µm and the sample was translated (in the z-direction) such that the laser focus was up to 1200 µm above and below the surface of the tissue, as shown in figure 3.13. As seen in the schematic of figure 3.13 a “+” value refers to the laser focus being inside the tissue whereas a “-“refers to the focus being above the tissue. The results presented in figure 3.14 clearly show that a reasonable ablation occurred when the laser focal point was either positioned inside the tissue or at the surface of the tissue. It was also observed that the ablation depth when the laser was focused at the tissue surface was similar to that when the focus was at + 400 µm (inside the tissue) of the focal point of laser. The deepest ablation (~180 µm) was measured for the focus positioned at the surface or at + 400 µm. In the +400 µm case the convergent beam is inside the tissue and the applied fluence is therefore sufficient to resect the tissue. For +800 and + 1200 µm the ablation depth decreased. When the laser focal point (a diameter of ~34.4 µm) was 400 µm above the tissue surface (~ 400 µm), a superficial ablation with a low ablation depth was observed. For – 800 µm a superficial ablation (~ 60 µm maximum depth) was observed and at – 1200 µm no ablation was observable. This is due to the diverging beam interacting at the tissue surface with a too low fluence for an efficient ablation process. In conclusion, the ablation depth has a
negligible change when the laser is focused at the tissue surface or inside the tissue up to a depth of 400 µm and the process works effectively in this regime. Wang et al. performed a focus variation laser machining inside a glass sample using 12 ps laser pulses [3.21]. The ablation efficiency was observed to reduce from the top of the glass surface to the bottom surface, and a large difference in ablation efficiency was noted when the laser focus was out of the Rayleigh range [3.22]. Figure 3.14 shows the 3D profilometric images of the laser ablated tissue zones at different focal position. The table 3.3 shows the average depth of the crater according to the position change in laser focal spot on tissue.

![Figure 3.13](image1.png)

Figure 3.13. Schematic of the focusing of laser beam in/out/on the surface of tissue. In these experiments the tissue sample was translated through the fixed focal position.

![Figure 3.14](image2.png)

Figure 3.14. 3D optical profilometric images of laser ablated zones with 10 J/cm² laser fluence with laser focal point adjusted in the tissue according to the movement of the z axis stage.
<table>
<thead>
<tr>
<th>1 kHz, 1030 nm (µm)</th>
<th>Mean depth (µm)</th>
<th>SD (µm)</th>
</tr>
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<tbody>
<tr>
<td>F+1200</td>
<td>133</td>
<td>±45</td>
</tr>
<tr>
<td>F+800</td>
<td>130</td>
<td>±29</td>
</tr>
<tr>
<td>F+400</td>
<td>180</td>
<td>±40</td>
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<tr>
<td>Focus (F)</td>
<td><strong>181</strong></td>
<td>±47</td>
</tr>
<tr>
<td>F-400</td>
<td>108</td>
<td>±27</td>
</tr>
<tr>
<td>F-800</td>
<td>59</td>
<td>±6</td>
</tr>
</tbody>
</table>

Table 3.3. Average depth of the crater according to the tissue position change based on laser focus

3.4.2. Tissue resection using 1 kHz, 10 kHz and 20 kHz PRR laser pulses

3.4.2.1. Tissue resection using 1 kHz

The initial tissue resection experiments were carried out at 1 kHz PRR. The temporal pulse-to-pulse separation for this PRR is 1 ms and an approximately 90% spatial pulse overlap was achieved by scanning the beam at 3.4 mm/s in laser scanning direction. Results for 5 different laser fluences are shown in figure 3.15 which shows the histology of laser resected colon and the corresponding surface profile. The build-up of successive pulses on the same surgical area of tissue can cause significant thermal and mechanical damage to the adjacent tissue due to shock wave and cavitation bubble formation (these effects are explained in chapter 2 section 2.2.4). However, by operating in the 1 kHz PRR regime (even with a high spatial pulse overlap) it was proposed to reduce these effects as the resection zone of tissue would have sufficient time for the heat to dissipate before the arrival of the next pulse. Therefore, the heat accumulation effect is negligible for 1 kHz operation. A detrimental effect, however, is that with lower repetition rate then the overall processing time is increased due to slower scan speeds used for a given spatial pulse overlap.
Figure 3.15. H&E stained images and corresponding 3D profilometric images of laser ablated zones with different laser fluences at a PRR of 1 kHz and 90% spatial pulse overlap. 11a) 12 J/cm² 11b) 10 J/cm² 11c) 8 J/cm² 11d) 6 J/cm² 11e) 4 J/cm²
The histology images are helpful for identifying tissue damage and ablation depth after the laser resection of colon tissue. Such histology results are used in literature to indicate the efficiency of the laser ablation [3.23-25]. The ablation depth and width of thermal damage was analysed in relation to applied laser fluence. An example image showing the width of thermal necrosis following ablation with 1030 nm laser is marked in figure 3.16a. The thermally damaged area is marked in the figure and in all the areas the necrotic tissue width is less than 50 µm. In the first three images of figure 3.15 (3.15a, 3.15b and 3.15c), the mucosal layer has been completely removed, while some of the submucosal area is also resected at the higher laser fluences (i.e. 10 and 12 J/cm²). As in figure 3.16a the histology images of five ablated craters shown in figure 3.15 were analysed to find the width of thermal necrosis which is consistent for all five laser fluences used (<50 µm). The necrotic tissue width in the histology images are measured using Aperio ImageScope software from Leica Biosystems. Figure 3.15 also show the depth over the ablated region but it should be noted that some shrinkage of the profilometric samples occurred due to the dehydration process. The depth of the laser ablated zones are indicated on the false
colour scale bars. These results confirm that the resection is controllable by adjusting the pulse energy and laser fluence. Increasing the laser fluence also increased the depth of the ablation and the relationship between average ablation depth and laser fluence is shown in figure 3.17.

Figure 3.17. The depth of tissue resection as a function of laser fluence applied on the inner lining of the colon tissue. The error bar represents the variation in the resection depth measurement found from different regions of the ablation crater.

The maximum average ablation depth of 1 kHz PRR with 107 μJ (12 J/cm²) is 309±43 μm. The volume of the crater can be calculated using the equation to find the volume of the approximately rectangular prism of the crater. This is the product of length, width and height of the crater and hence the calculated ablation rate (volume/time taken for ablation) at 107 μJ is approximately 0.21 mm³/minute. These 3D profilometric samples underwent a critical point drying (CPD) mechanism after glutaraldehyde fixation. CPD is a method for drying the sample.

The simple columnar epithelial cells are easily removed by applying relatively low laser fluence of 4 J/cm² as seen in the figure 3.15e. This laser fluence is also capable to generate plasma on the surface of the colon tissue to perform the surgical process. The submucosa is bounded with muscularis mucosa and muscularis externa layers. In the histology images, it is evident that smooth muscle tissue layers are also present in the submucosa layer. The muscle layers are more resistive to the interaction with laser pulses, which is evident from figure 3.15c. In figure 3.15c, the depth of ablation is limited by the
muscularis mucosa and generates a relatively uniform ablation pattern with 8 J/cm² applied laser fluence.

For comparison to previous similar work in 2016, Kaushik et al. used a 100 µm thick porcine vocal fold tissue to investigate the ultrafast laser ablation characteristics. A laser with a pulse width of 1.5 ps, PRR of 303 kHz and wavelength of 776 nm was used to ablate the tissue with a maximum fluence of 7.8 J/cm². The laser was transmitted through a Kagome fibre optic probe, which was used to generate a lissajous pattern on the tissue surface at a frequency of approximately 1 kHz. Laser ablation on the tissue has a depth and width of around 43 and 40 µm respectively at a pulse energy of 1.2 µJ. However, no histology results are provided with this work so it is not possible to evaluate the impact on underlying tissue in a clinically relevant context and in particular assess the extent of the thermally damaged region [3.26]. Lingfei et al. conducted a study of near infrared femtosecond laser ablation in human enamel and dentin to find the ablation threshold. They have used different laser fluences ranging from 1.13 to 3.68 J/cm² with 1 kHz PRR. They found negligible effect of heat and shockwave in this ablation [3.27]. Earlier research has shown a significantly larger width of thermal necrosis induced by conventional surgical devices such as electrocautery (456 µm) [3.28], harmonic scalpel (690 µm) and CO₂ laser (300 µm) shown in figure 3.18. CO₂ laser creates poor haemostasis compared to electrocautery or harmonic scalpel [3.29]. The analysis of thermal damage in small bowel surgery based on electrocautery, CO₂ laser, and Nd: YAG laser reported a maximum thermal damage of 15 mm, 4 mm, and 4.2 mm respectively, similarly the use of harmonic scalpel in haemorrhoidectomy creates a maximum thermal damage of 1.5 mm [3.30-31]. In contrast, the histology results presented here, as shown in figure 3.16a demonstrate that a thermally damaged region of less than 50 µm is achievable with ps laser resection of porcine colon.
Figure 3.18. The blue line shows the thermal damaged region present when using harmonic scalpel (A) and (B) CO$_2$ Laser for tongue resection [3.29].

These results obtained from the galvanometer study clearly demonstrate that precise tissue ablation with controllable depth and limited necrosis to the surrounding tissue was possible using 6 ps pulses with 1 kHz PRR.

**3.4.2.2. Tissue resection using 10 and 20 kHz**

The total time taken for a given surgical procedure is of critical importance and ideally should be minimized where possible. The ability to operate the laser at higher repetition rate opens up the potential for faster processing as faster scanning speeds can be used for a given spatial pulse overlap. The histology results of tissue ablation using 10 kHz and 20 kHz PRRs are presented in figure 3.19. Figure 3.19 a1, a2 and a3 represents 10 kHz PRR ablation with 12, 10 and 8 J/cm$^2$ laser fluence operation respectively. Figure 3.19 b1, b2, b3 and b4 represents 20 kHz PRR ablation with 12, 10, 8 and 6 J/cm$^2$ laser fluence operation respectively at 90% pulse overlap. Unfortunately, three ablation patterns were missed by the pathologist during cutting of the tissue and hence there is no histology for 10 kHz 6 and 4 J/cm$^2$ and 20 kHz 4 J/cm$^2$. Due to the limited availability of animal tissue these results were not repeated. Nevertheless, with the presented data it was possible to assert some meaningful insight into higher PRR operation.

Although it was observed that with the highest laser fluence operation in 10 kHz PRR ablation an ablation crater was created the overall precision was less than that achieved with the 1 kHz operation and disruption of tissue into the muscle layer can be observed.
(figure 3.19a1). The other areas machined at 10 and 20 kHz showed evidence of higher thermal damage and no well-defined crater. This is due to the rate of interaction of the higher number of laser pulses compared to 1 kHz ablation. In this experiment, 90% pulse overlap percentage has been used for the pulse overlap strategy to keep same total energy applied to the tissue surface. However, the temporal separation of adjacent pulses for 10 kHz and 20 kHz is (100 and 50 µs) respectively 10 and 20 times shorter than for the 1 kHz. Although the pulses are spatially separated to the same degree as for 1 kHz, the shorter time gap between successive pulses results in detrimental thermal effects. This is discussed in more detail in section 3.5 where methods to alleviate these issues are investigated. The 10 kHz ablation was repeated using carbide laser with 13 and 18 J/cm² laser fluence (46 and 33 µJ pulse energy) and 6 ps pulse width. A reasonable result was achieved with a thermally damaged region around 100 µm (presented in section 3.5.1).
Figure 3.19. H&E stained images of laser ablated zones with different laser fluences. a1, b1) 12 J/cm$^2$, a2 b2) 10 J/cm$^2$, a3 b3) 8 J/cm$^2$ and b4) 6 J/cm$^2$. All “a” sample were processed at 10 kHz and all “b” samples were processed at 20 kHz
It is evident from the 10 kHz and 20 kHz ablation that tissue damage occurred due to photodisruption and heat accumulation. Tissue ionization is the main factor determining the efficiency of plasma mediated ablation but photodisruption is another kind of surgical effect based on mechanical disruption [3.32]. The plasma mediated ablation and photodisruption are described in detail in chapter 2. The plasma generation, shock wave, cavitation and jet formations effects are happening in a tissue at different times during ultrashort pulse laser interaction of tissue [3.33]. In a laser pattern with high percentage of pulse overlap the secondary effects are significant and it reduces the efficiency of the plasma mediated ablation.

In conclusion, the 10 kHz ablation is not reliable in laser fluence lower than or equal to 12 J/cm$^2$ operation with higher pulse overlap and the high pulse energy operation shows less precision than with the 1 kHz results. No effective resection was achieved with 20 kHz PRR ablation and 90% pulse overlap with damage to the mucosa and submucosa due to the secondary or induced effects of plasma generation or optical breakdown. The heat dissipation capacity of the colon tissue is compromised in higher PRR where the time between subsequent pulses is reduced (to 100 and 50 µs). In addition to that the thermal conductivity of the colon is very low (0.556 W/m.K) [3.34]. However, to investigate further the higher PRR operation with higher laser fluence (>12 J/cm$^2$), the ablation has been performed with 18 J/cm$^2$ and 13 J/cm$^2$ with low pulse energy. By using a modified spiral scanning strategy with the Carbide laser system a more efficient surgical effect has been observed (these results are presented in section 3.5).

### 3.4.3. Tissue resection using different pulse overlaps

In this experiment the pulse separation in laser scanning direction and laser line separation direction are same. The figure 3.20 a1, b1 c1 and d1 shows the 20 kHz PRR ablation of tissue with 88.5 µJ pulse energy (fluence of 10 J/cm$^2$) and pulse overlap percentage of 94, 92, 90 and 88 respectively in both the laser line spacing and laser scanning direction. Figures 3.20 a2, b2, c2 and d2 are the corresponding 3D profilometric images. For all samples processed using the above parameters a bulge, raised tissue was observed in the region where the laser was applied, which is also evident in the profilometric images. This is due to the formation of cavitation bubbles (or gas bubbles) inside the tissue.

It should be noted that in this experiment the size of the laser pattern was 2 mm$^2$ (The laser ablated area is increased to help the pathologist to avoid un-ablated area sectioning),
so the total number of pulses interacting with the surgical zone was approximately four times greater than the previous experiments. The secondary effects of shockwaves and cavitation bubbles are significant in this experiment, which propagate to the adjacent tissue surrounding the surgical zone causing rupturing of the tissue [3.35]. The mechanical rupturing effects are evident in the histology results showing in figure 3.20 a1. If the tissue rupturing was based on shock waves (which is more significant) then it transmits through mucosa and submucosa creating mechanical damage. On the other hand, if cavitation effects were more significant in tissue damage, then the cavitation bubbles were concentrated heavily in the top layer and the effect is limited in the area of interaction of the pulse (shown in figure 3.21). The effect of cavitation bubble and shockwaves in tissue ablation is explained in detail in chapter 2 section 2.2.4.
Figure 3.20. H&E stained images and corresponding 3D profilometric images of laser ablated zones with different laser pulse overlap percentage with 88.5 μJ (10 J/cm²) and 20 kHz PRR. Pulse overlaps are a) 94%, b) 92%, c) 90% and d) 88%
The efficiency of the plasma mediated ablation is compromised because of cavitation bubble generation. The plasma formation at the laser focal point is obstructed by cavitation bubble formation when the following laser pulse is arriving at the tissue surface. This effect can be observed when the time interval between successive laser pulses are shorter than the bubble life time which is reported to be less than 1 µs [3.36] near the optical breakdown in water. The bubble life time will change according to the applied pulse energy above the ablation threshold value. In 2011 Jayasinghe et al. demonstrated the change in cavitation bubble life time according to the applied pulse energy in different samples, including deionized water and fruit fly embryo. It has been observed cavitation bubble life time greater than 50 µs when using 40 µJ pulse energy [3.37]. Therefore, the cavitation bubbles are created at higher repetition rates of the laser where the temporal separation of the laser pulses is reduced (for a PRR of 20 kHz the time between successive pulses is 50 µs compared to 1 kHz operation where the time between pulses is 1 ms). The gas bubbles are preserved in the histology sample because the tissue is fixed in formalin after the laser processing. The complex dynamics of the cavitation bubble formation is illustrated in [3.35].

The effect of cavitation bubbles leading to raised tissue in the surgical zone was further demonstrated for 40 kHz PRR for a fluence of 10 J/cm². Figure 3.22 represents 3D...
profilometric images of 40 kHz ablation pattern with a pulse overlap percentage of 94, 92, 90 and 88 corresponding to figure 3.22 a1, a2, a3, and a4. A bulge in the surgical zone is obvious in all the images similar to the 20 kHz ablation pattern, due to the aforementioned expansion of gas bubbles inside the tissue. In conclusion, the tissue resection procedure using PRR of 40 and 20 kHz did not create well-defined craters in the tissues and showed evidence of detrimental thermal and cavitation bubble effects during the ablation process using 10 J/cm² laser fluence. These parameters therefore do not provide a reliable and efficient surgical strategy for tissue resection.

Figure 3.22. 3D profilometric images of laser ablated zones using a PRR of 40 kHz (a1, a2, a3, a4) with laser fluence of 10 J/cm². The pulse overlap percentage a1) 94%, a2) 92%, a3) 90% and a4) 88%
3.5. Porcine Tissue resection using CARBIDE laser

3.5.1. Tissue Resection using 90 % Pulse overlap

Using the Carbide laser (6 ps pulse width), a 10 kHz PRR laser ablation has been performed with 18 (46 µJ pulse energy) and 13 (33 µJ pulse energy) J/cm² laser fluence, 90 % pulse overlap in the laser scanning direction and laser line separation with a pattern size of 2 mm diameter (18 µm spot size). The total time taken to complete one iteration of ablation pattern was 97 s. The ablation depth and width of thermal damage were analysed in relation to the applied laser fluence. Figure 3.23 shows the histological pattern of laser resected colon and the corresponding surface profile. The mucosal layer has been completely removed with a fluence of 13 J/cm², while some of the submucosal area is also resected at the higher laser fluence (i.e. 18 J/cm²), this is evident in the histology pattern. Figure 3.23 also shows the depth over the ablated region but it should be noted that some shrinkage of the profilometric samples occurred due to the glutaraldehyde fixation process. The average depths of 550±182 and 440±165 µm are achieved at 18 and 13 J/cm² laser fluence respectively. The depth of the laser ablated zones are indicated on the false colour scale bars. A thermally damaged region (~85 µm) and fine depth control of ablation using the pulse energies 46 and 33 µJ are demonstrated, these pulse energies are relatively low compared to 107 to 70.5 µJ pulse energy ablations presented in section 3.4.2.2.

![Figure 3.23](image)

Figure 3.23. H&E stained images and corresponding 3D profilometric images of laser ablated zones with different laser fluence. a1, a2) 18J/cm², b1, b2) 13J/cm²
3.5.2. Tissue resection with reduced pulse overlap

The next set of experiments were performed with low pulse overlap percentage in the laser scanning direction (i.e. higher spatial pulse separation) to avoid the shock wave and cavitation effect. To achieve a high pulse separation, the scanning speed of the galvo scan head was adjusted according to the temporal pulse separation and PRR. Dr. Rainer Beck, working in the same research group, introduced the idea to reduce the pulse overlap in the scanning patterns to improve resection at higher PRR. In Dr Beck’s initial work, the results demonstrated that well-defined craters with low thermal damage regions could be achieved by spacing out pulses spatially using an appropriate scanning strategy. The following work reports experiments conducted to confirm the approach proposed and demonstrated by Dr Beck. In 2007, Henning et al. demonstrated femtosecond laser based vocal fold tissue ablation with high spatial separation of pulse (10% overlap) [3.38], which is explained in detail in section 2.3 of chapter 2.

When low overlap percentage of pulses in a raster scan pattern (figure 3.2) were attempted with the Trumpf laser repeated errors caused the laser to turn off while performing the ablation on tissue. This arose due to the rapid, repeating action of switching OFF the laser during the flyback time then ON for the ablation. It was therefore decided to perform outward spiral patterns to ablate the tissue (see figure 3.11). In this case there is no laser turn off time (as described in section 3.2.5.1) during ablation as the spiral is a continuous machining pattern. The following experiment using a spiral pattern were performed using the Light Conversion CARBIDE laser (figure 3.4) as the Trumpf laser had to undergo significant maintenance work.
In the first case, an outward spiral pattern has been generated with 70% pulse overlap in the laser scanning direction and 90% pulse overlap in the laser line separation (figure 3.11) with a 2 mm diameter. The PRR rate was 20 kHz and the fluence was 13 J/cm². In this experiment the mechanical effects are significant and the ablated tissue zone exhibits obvious mechanical damage, which is shown in figure 3.24 a1. The above parameters were repeated with 50% pulse overlap in laser scanning direction and again observed the tissue damage shown in figure 3.24 b1. In each of the cases the absence of cavitation bubbles has been noticed even though the laser is being operated at high PRR (20 kHz).

In the above cases the spiral scanning patterns are repeated three times on the targeted tissue surface. In 2003, Vogel and Venugopalan proposed a scanning strategy to avoid heat accumulation during laser ablation. The technique was to lengthen the spacing between laser pulses used for the tissue resection [3.39]. By separating out the pulses spatially in the direction of the scanning and using repeated scans to ablate deeper into the tissue, the effects of cavitation bubbles can be minimised (although there is still obvious thermal/mechanical damage due to the relatively high pulse overlap used).
3.5.3. **Tissue resection with 0% and 20% pulse overlap**

Significant reduction in the total surgical procedure time can be achieved by increasing the laser pulse repetition rate. Although the 1 kHz operation showed well-defined craters with minimal thermal damage it is important that successful strategies using higher PRR are investigated. It has been observed that the plasma induced secondary effects are dominant in higher pulse repetition rate operation with higher pulse overlap. Although the separation of 70 and 50% reported in the previous section showed that the cavitation bubble effect could be alleviated, the results still showed significant mechanical/thermal damage to surrounding tissue. The next set of experiments were performed using 0% pulse overlap in the scanning direction and 90% pulse overlap in the laser line separation direction in an outward spiral pattern (see figure 3.11). The PRR was 20 kHz and the fluence was 13 J/cm². To avoid the heat accumulation effects the pulses have 0% overlap (i.e. completely separated spatially) in the scanning direction. In this scenario the following laser pulse does not interact with the previous laser pulse. Therefore, the damage due to heat accumulation is negligible in this operation. To achieve 0% pulse overlap in the laser scanning direction the galvo scanhead speed was set at 360 mm/s for 20 kHz PRR used. The laser ablated tissue using this parameters are shown in figure 3.25. To achieve a 90% pulse overlap in line separation, the outward spirals are separated at a distance of 1.8 µm.
Figure 3.25. H&E stained images and 3D profilometric images of laser ablated zones with a 13J/cm² laser fluence, 20 kHz PRR, 0% pulse overlap in the direction of laser scanning, 90% overlap in laser line separation and a different number of iterations of scanning patterns. a) 10 times, b) 8 times, c) 6 times and d) 4 times.

The white regions on the 3D profilometric images are missing data points which is a limitation of the device where it becomes difficult to receive reflected light signals from
the sample areas which have a high slope angle or large height differences in the ablated region [3.40]. The rapid expansion of plasma leads to the formation of shock waves and cavitation bubbles in the tissue. The development of a large plasma pressure gradient, due to the operation of high pulse overlap interaction, is the reason for this plasma expansion [3.41]. The separation of pulses limits the generation of high plasma pressure gradient in the laser focused region. In 2011, Vogel and Venugopal reported that the plasma pressure is proportional to the product of the temperature of the free electron and free electron density [3.42]. The separation of laser pulses therefore aids the reduction of the temperature and density of free electrons. Thus, using 0% pulse overlap in the laser scanning direction, with 13 J/cm² fluence helps to create an efficient surgical effect on porcine tissue surface. The difference in correlation of depth between profilometric and histology images were evident figure 3.25, this is due to the cutting disparity at the centre of the crater during histology.

Figure 3.25 represents the H&E stained images and corresponding 3D profilometric images of laser ablated zones using 20 kHz PRR. Figure 3.25 a, b, c and d correspond to the number of times the laser pattern repeated 10, 8, 6 and 4 respectively. A 33 µJ pulse energy was used to create this efficient surgical effect without cavitation bubble formation and no evidence the mechanical damage seen previously (figure 3.24). The average ablation depths are proportional to the total energy applied in each surgical zone. The average ablation depths were calculated using the method described in section 3.3. From the histology results it is evident that the ablation is confined in the width of the spiral pattern and the depth of the ablation is not more than the submucosa layer. The clinical experts Prof. David Jayne and Dr. Nicholas West confirmed that this surgical modality is a better solution to early stage cancers and polyps generating in the mucosa and submucosa. The maximum thermal damage in this ablation is < 60 µm, which is evident in the H&E stained images, while the sides of the resected tissue area were well defined with zero or negligible thermal damage.

The volume of the crater was calculated by using the equation to find the volume of a cylinder (shown in figure 3.26). The laser resected region in the tissue can be approximated to the shape of a cylinder. The ablation rate was calculated by equation 3.6,

\[
\text{Ablation rate} = \frac{\text{Volume}}{\text{Total time taken for ablation}} \quad (3.6)
\]

The time taken to perform a 20 kHz outward spiral pattern with 2 mm diameter and 0% pulse overlap is 4.84 s for a single iteration (described in section 3.2.5.1). Therefore, the
total processing time for laser pattern iterations of 10, 8, 6 and 4 times takes 48.4, 38.72, 29.04 and 19.36 seconds respectively. The average ablation depth of the surgical zones shown in figure 3.25 a, b, c and d were calculated as 761±256, 702±169, 630±147, 624±185 µm respectively. The calculation method is described in section 3.3.

![Diagram of a cylinder](image)

Figure 3.26. Schematic of a cylinder

\[
\text{Volume of the cylinder (V)} = \pi r^2 h
\]

(3.7)

The ablation rates for the 0% pulse overlap pattern is presented in figure 3.27. The maximum ablation rate (Volume/total ablation time) of 6 mm³/minute is achieved for the lowest number of iterations of the spiral pattern. It is evident from the graph that ablation rate is decreasing according to the increase of number of iterations of spiral pattern. This is due to the lack of re-positioning of the sample along the laser propagation direction (z-direction) during the ablation. In order to keep the laser spot size the same on the tissue surface then the samples should be translated upwards by a distance approximate to the ablation depth achieved with the previous iteration. This would ensure constant fluence for each iteration. However, as this might be hard to achieve in a practical surgical context the effect of not moving the sample was investigated as shown in figure 3.28. The spot diameter becomes approximately double around 300 µm below the laser focus. Therefore, a reduction of ablation rate was observed according to the number of repetitions of the spiral pattern.
Figure 3.27 Ablation rate versus number of iterations of the spiral pattern using 13 J/cm$^2$ at 20 kHz and 0% pulse overlap in the laser scanning direction.

Figure 3.28. Illustration of the effect on ablative removal of a) maintaining the tissue in same position and b) translating towards the incoming beam to adjust the focus.
The total length of the spiral pattern is an important factor to find out the total time taken to complete one iteration of the pattern. The calculation has been presented in section 3.2.5.1. The total length of the 2mm diameter outward spiral pattern used was 1743 mm.

The total number of pulses is the ratio of total length to pulse separation distance. Consequently, the total number of pulses in the pattern was 96833 and the rate of energy interacting with the tissue was 0.65 J/s, the calculation is presented in section 3.2.5.1. The total number of pulses interacted with tissue in 20 kHz, 90% pulse overlap (in laser scanning direction and line separation direction) of 2 mm² raster scan pattern was 345744 (Shown in figure 3.20c1), which is approximately 3.5 times greater than the number of pulses in 0% overlap of 2 mm diameter spiral pattern. In the 90% pulse overlap raster scan pattern presented on figure 3.20c1, the laser fluence was 10 J/cm² and in 0% pulse overlap spiral pattern the applied laser fluence was 13 J/cm². The 90% pulse overlap is more prone to high thermal and mechanical damage via heat accumulation and secondary effects of plasma formation of successive laser pulses. For 0% overlap minimal thermal damage is observed because of the non-interaction between successive pulses, even though the laser fluences are similar.

As a comparison a 20% pulse overlap in the scanning direction was investigated by setting the galvo scanning speed at 288 mm/s for 20 kHz (shown in figure 3.29). To achieve a 90% pulse overlap in line separation, the outward spirals are separated at a distance of 1.8 μm. The time taken to perform 20% temporal pulse overlap outward spiral pattern with 2 mm diameter in single iteration is 6.05 s.
Figure 3.29. H&E stained images and corresponding 3D profilometric images of laser ablated zones with 13J/cm² laser fluence, 20 kHz PRR, 20% pulse overlap in direction of laser scanning and a different number of iterations of scanning patterns. a) 10 times, b) 8 times, c) 6 times and d) 4 times.
The total time of processing for laser pattern iterations of 10, 8, 6 and 4 times takes 60.5, 48.4, 36.3 and 24.2 seconds respectively. The average ablation depth of the surgical zones shown in Figure 24 a, b, c, and d and were calculated as 841±214, 775±163, 610±42, 519±107 µm. The ablation rate was calculated by using equation 3.6. Figure 3.30 shows the ablation rates for 20% pulse overlap operation with 13 J/cm² fluence at 20 kHz. From the histology images it is evident that the resection is limited to the submucosa area, which again is helpful for the removal of early stage cancers. It has been demonstrated that a 20 kHz PRR with 0% pulse overlap and 20% pulse overlap provides an excellent scanning strategy to remove neoplastic tissues in the inner lining of the colon. The observed maximum thermal damage is <60 µm in these experiment.

With 20% pulse overlap the maximum and minimum ablation rates achieved were 4 and 2.6 mm³/minute when using the 4 and 10 iterations respectively. The ablation rate at 20% overlap is lower than that compared to the 10% overlap, which may be due to the scanning speed and total tissue resection time. The scanning speed of the 0% overlap pattern was 360 mm/s and 20% overlap pattern was 288 mm/s. The 288 mm/s scanning speed for a 20% pulse overlap pattern also causes pulse to pulse interaction. In 2016 Kerse et al. demonstrated the ablation of rat brain tissue using ultrafast burst pulses with a maximum ablation rate of 2 mm³/ minute [3.43]. The present work demonstrated a maximum ablation rate of 6 mm³/ minute (using 13 J/cm² laser fluence, 20 kHz PRR and 0% spatial pulse overlap) which has obvious advantages when considering the reduction of surgical time. Studies have been reported related to the ablation rate of ultrashort laser pulse ablation in hard tissue [3.44-45]. In 2012 Schelle et al. demonstrated a maximum ablation rate of 7.69 and 6.41 mm³/minute in dentin and enamel hard tissue respectively, with a maximum laser power of 10 W, 500 kHz PRR and pulse width of 8 ps [3.46]. They also proved that the ablation rate can be scaled up to 47.5 and 39.8 mm³/minute for dentin and enamel respectively by using a maximum power of 50 W with the other laser parameters remaining the same. The ablation rate is very high because of the repetition rate used and the nature of the tissue, also the sample was adjusted during the ablation time along the optical axis according to the number of iterations of patterns. In contrast for soft tissue, Kaushik et al. achieved a material removal rate of 0.26 mm³/minute in a porcine vocal fold microsurgery [3.26], explained in detail in section 3.4.2.
Figure 3.30. Ablation rate versus number of iterations of the spiral pattern using 13 J/cm$^2$ at 20 kHz and 20% pulse overlap in the laser scanning direction.

3.6. Conclusions

An efficient surgical process for colon tissue resection with negligible thermal and mechanical damage has been demonstrated using a 1030 nm ultrafast laser with 1 kHz PRR and 6 ps pulse width. The maximum average depth of 309±43 µm achieved at 12 J/cm$^2$ laser fluence and a minimum average depth of 122±7 µm achieved at 4 J/cm$^2$ laser fluence. It was shown that the ablation depth is directly proportional to the applied laser fluence in fresh colon tissue resection. However, for 1 kHz operation the rate of ablation was found to be relatively low, approximately 0.21 mm$^3$/minute, for the maximum average ablation depth achieved. The laser processed tissues were analysed using histology results and 3D optical profilometric results to monitor the shape, depth, thermal and mechanical damage of the surgical zone. The mucosa and a part of submucosa was resected efficiently using 1mm$^2$ raster scan patterns with 90% pulse overlap in both the laser scanning direction and laser line separation.

In 10 kHz and 20 KHz PRRs, the 1mm$^2$ raster scan patterns were performed on the porcine colon surface with 90% pulse overlap with different laser fluences. The heat accumulation effect and mechanical effect have been observed in most of the patterns used. This is due to reduced time between successive pulses (relatively high pulse energy compared to 10 kHz ablation presented in section 3.5.1) causing detrimental interactions. Heat accumulation and mechanical rupturing due to shockwave propagation to the adjacent tissue was also observed in the H&E stained images. For 12 J/cm$^2$ laser fluence
of operation at 10 kHz produced a well-defined ablation pattern but mechanical damage is present in the submucosal area. However, the laser resected sections below 12 J/cm² (at 10 kHz) exhibit higher thermal damage. To prove the repeatability well defined ablation patterns with higher laser fluences (relatively low pulse energy) at 10 kHz PRR, 13 J/cm² (33 µJ) and 18 J/cm² (46 µJ) spiral patterns were ablated on the tissue surface. The mucosal tissue has been completely removed with a fluence of 13 J/cm², while some of the submucosal area is also removed at the higher laser fluence (18 J/cm²). The average ablation depths of 550±182 and 440±165 µm are achieved at 18 and 13 J/cm² laser fluence respectively. The rate of ablation was 1.06 mm³/minute for 18 J/cm² laser fluence operation. In conclusion, plasma induced ablation of tissue in 10 and 20 kHz PRR with high pulse overlap and high pulse energy was interrupted by thermal and mechanical effects. In 10 kHz PRR, more thermal and mechanical effects were visible for ablation with ≤ 12 J/cm² laser fluence. This may be due to the dominancy of shock wave generation and associated heat for the applied pulse overlap and pulse energy. Further studies on 10 kHz ablation with higher laser fluences (>12 J/cm²) and lower pulse energy has been presented in chapter 4. The results at 1 kHz confirm that the resection is controllable by adjusting the pulse energy and laser fluence with a minimal heat affected zone and hence demonstrates the viability for precision tissue resection.

The next set of experiments were performed on the tissue surface with 10, 20 and 40 kHz PRRs with 10 J/cm² fluence and different percentage of pulse overlap (94% to 88%). The histology results clearly demonstrated the persistent gas bubbles in the tissue due to cavitation bubble formation. This methodology is widely accepted as photodisruption with minimal thermal and mechanical damage to the adjacent tissue. In conclusion, an efficient plasma mediated ablation is not possible with high overlap percentage in 10, 20 and 40 kHz PRR operation at 10 J/cm² fluence and 88.5 µJ pulse energy. The plasma induced secondary effects are significant in this regime that reduces the efficiency of surgery.

With regards to a potential application of picosecond laser resection in real surgery the total resection time and the tissue removal rate are critical. The spatial separation of pulses were increased and experiments performed to avoid gas bubbles, mechanical rupturing and heat accumulation in the surgical zone. In the initial experiments, the pulse overlap percentage was reduced to 70% and 50% compared to the previous experiments done with 10, 20 and 40 kHz PRR. The spiral scan patterns were performed on the tissue surface and that generated a thermally damaged surgical zone and the ablation was not
well-defined or controlled. On the other hand, the 20% and 0% temporal pulse overlap scanning strategy performed an efficient resection of tissue with minimal thermal and zero mechanical damage. The highest ablation rate of 6 mm³/minute was observed in 0% pulse overlap. This surgical modality has the potential capability to resect the early stage lesions and polyps in the mucosa and submucosa, which is evident in the histology results. In this study, the paramount importance of ultrafast laser pulses in colon tissue ablation has been demonstrated with a necrotic tissue width of <60 µm.

3.7. References


Chapter 4

515 nm picosecond laser resection of colon tissue in a porcine model

4.1. Introduction

The application of a 515 nm ultrashort pulse picosecond laser in colon tissue resection is presented in this chapter. The picosecond laser processing strategy with high and low pulse overlap and different fluences are used to resect the colon tissue of a porcine model. The relation between effectiveness of tissue ablation and the laser parameters, such as repetition rate, pulse overlap and laser fluence will be presented. In 1030 nm laser ablation, the coupled plasma energy to targeted tissue contribute to the effective surgical process, whereas the photodisruption effects such as shock wave, cavitation bubble and jet formation limit the efficacy of colon tissue resection for precise shape and depth. The plasma mediated secondary effects supports mechanical rupturing of tissue with unwanted thermal effects in the surgical zone. The properties of generated plasma on the tissue surface depends on different factors such as the characteristics of the target, laser pulse width, and irradiance [4.1]. In this chapter, the tissue resection based on plasma generation (using 515 nm and 6 ps laser pulse) and its associated effects are also investigated.

4.2. Experimental Setup

The tissue excision experiments were carried out using an Yb: YAG regenerative thin disk laser amplifier (Trumpf True Micro 5X50) that produces laser pulses with a duration of approximately 6 ps at wavelength of 515 nm (Second Harmonic). An electronically controlled beam switch is used to select 515 nm with an output power of 24.5 W and a maximum pulse energy of 61.33 μJ. The pulse repetition rate of this laser system controlled by a computer based acousto-optic modulator (AOM) and the maximum pulse repetition rate is 400 kHz with a beam quality ($M^2$) value of <1.3 and a focal spot diameter of approximately 20 μm. A galvanometer scan head is employed to focus the laser beam to the tissue surface for precise resection. The schematic of the setup is shown in figure 3.1 of chapter 3. The focused spot size of the laser was measured using a scanning slit beam profiler (BeamMap2, Data Ray Inc.). The function of the scanning slit beam profiler is explained in section 3.2.3 of chapter 3. The waist size of the focused beam was measured to be approximately 20 μm in diameter (shown in figure 4.1 & 4.2), with an F-theta lens of focal length 160 mm. This was smaller than the focal spot diameter (34.4
μm) for the 1030 nm wavelength laser. Table 3.1. shows the parameters related to Trumpf TruMicro 5x50 laser.

Figure 4.1. Two dimensional (a) and three dimensional (b) isometric plots of a picosecond 515 nm laser beam at the colon tissue surface

Figure 4.2. Horizontal and vertical cross section of the beam waist diameter at focus

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>515 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse Repetition Rate</td>
<td>1 to 400 kHz</td>
</tr>
<tr>
<td>Pulse Energy</td>
<td>Up to 61.33 μJ</td>
</tr>
<tr>
<td>Pulse Width</td>
<td>6 ps</td>
</tr>
</tbody>
</table>

Table 4.1. Parameters of Trumpf TruMicro 5x50 laser (515 nm)
4.2.1. Laser pulse energy and laser fluence

The laser ablation on porcine colon tissue was carried out for different samples. On each sample five separate areas were ablated with the same scanning pattern but with different laser pulse energies. 1mm$^2$ raster scanned area was ablated (similar to that used for 1030nm shown in figure 3.2 and 3.3) for 1 kHz and 10 kHz pattern and 2mm$^2$ raster scanned area was performed for histology samples of 20 kHz. The pulse repetition rates investigated initially were 1 kHz, 10 kHz, 20 kHz and 40 kHz. The laser line spacing was set at 2 µm to achieve 90% pulse overlap in the laser line direction of the raster scan pattern for a focused laser spot diameter of 20 µm. The scanning speed is varied according to the PRR to achieve 90% pulse overlap in the laser scan direction. The laser pulse energy was set at 58.57, 47.6, 36, 24.17 and 13.27 µJ; the corresponding laser fluences were approximately 19, 15, 11, 8 and 4 J/cm$^2$. The aim of using these altered fluence values was to examine the effect on ablation depth and the width of damage. An additional set of experiments were performed with 18, 13 and 5 J/cm$^2$ laser fluences with 90% pulse overlap with three iterations to see the effect of ablation depth and tissue damage.

Initially, most of the experiments were performed with 90% pulse overlap. However, some patterns with change in pulse overlap is also performed. In these experiments the pulse overlap was varied from 94% to 80%, using a pulse energy of 47.6 µJ (15 J/cm$^2$) to differentiate the effect of cavitation/mechanical effects (tissue damage) and the ablation depth. These experiments were performed in the 20 and 40 kHz PRR regimes.

In the next set of experiments, 1 mm$^2$ raster scan patterns were performed for 3D profilometric measurement and 2 mm$^2$ patterns were performed for H&E staining. The pulse overlap percentage in the laser line direction and laser scanning directions were 90% and 97% respectively. Laser pulse energy was set at 61.33, 57.9, 54.82, 51.05, 47.6 and 43.85 µJ; the corresponding laser fluence was approximately 19.5, 18, 17, 16, 15 and 14 J/cm$^2$. These experiments carried out in 10, 20 and 40 kHz PRR regimes.

The final set of experiments were performed with 0% and 20% pulse overlap in the laser scanning direction and 90% pulse overlap in laser line separation direction. The outward spiral scan pattern was performed (as shown in figure 3.11 of chapter 3) on the tissue surface with repeated iterations of the patterns to examine the change in crater depth and thermal damage. These experiments are carried out using a laser fluence of 13 J/cm$^2$. 
4.2.2. Preparation, positioning and Analysis of tissue samples

Preparation of the porcine colon sample after the postmortem of the euthanized pig is explained in detail in section 3.2.4 of chapter 3. The positioning of the tissue samples in Aerotech stages and the methodology to find focus was also explained in section 3.2.4 of chapter 3. An Alicona Infinite Focus (IF) profilometer is used to analyse the laser ablation profile of tissue specimen providing high resolution analysis via an optical 3D micro coordinate measurement system. The profilometric and histology analysis are discussed in section 3.3 of chapter 3.

4.3. Colon tissue resection using 90% pulse overlap

4.3.1. Tissue resection using 1 kHz PRR at 515 nm

In low repetition rate tissue resection, any heat energy deployed by individual laser pulses diffuses out of the focal volume before the succeeding pulse [4.2]. In other words, any temperature rise generated in the laser irradiated region, as a consequence of pulse interaction with sample (the temperature before plasma threshold), has sufficient time to return to a steady temperature state before the next pulse arrives [4.2]. This methodology is used to avoid heat accumulation in the surgical zone with a temporal pulse separation of 1 ms in 1 kHz PRR operation. This ablation regime has produced surgical zones with minimal/negligible thermal damage with well-defined ablation craters in hard and soft tissue ablation [4.3-4]. The 1 kHz PRR experiments were performed with 90% pulse overlap in the laser scanning direction and the laser line separation direction. This has a consequence of a longer total laser irradiation time (250.25 s) in 1 kHz ablation compared to tissue resection based on higher repetition rates. Figure 4.3 represents the 3D profilometric images of the laser ablated tissue region and histology images of tissue region resected at 1 kHz with a 90% pulse overlap for five different fluences.

It has been noticed that the maximum thermal damage was ~80 µm (shown in figure 4.4) in the surgical zone with an applied laser fluence of 19 J/cm². The selection of applied pulse energy/laser fluence is an important factor for laser ablation even if the 1 kHz PRR pattern gives enough time to avoid heat accumulation. However, the high laser fluence/pulse energy has the capacity to damage the tissue.

In figure 4.3 b1 and b2 the applied laser fluence was 19 and 15 J/cm² respectively, but the histology image shows that the 15 J/cm² ablation crater is slightly deeper than 19 J/cm² ablation crater. This can arise due to misalignment in positioning the centre of the
crater while slicing the tissue by the pathologist. The 3D pictures clearly demonstrate that the ablation depth varies according to the applied laser fluences. Figure 4.3, a4/b4 and a5/b5 represents the tissue resected regions with lowest laser fluences of 8 and 4 J/cm² respectively. The profilometric image in figure 4.3 a3 has small depth compared to the same laser parameter applied corresponding histology image. This is due to the uneven nature of the tissue surface and that induces a shift in the beam waist to the tissue surface. The 3D profilometric images shows a flat bottom surface with well-defined edges and clean ablation, the H&E stained images shows a clear ablation of the mucosal layer. Using 8 J/cm² laser fluence with 1 kHz PRR the mucosal layer is completely removed. However, the aim of this work was to develop an ablation strategy to resect the first two inner layers of the colon (mucosa and submucosa), where the early stage cancer arises. The calculated maximum ablation rate was 0.1 mm³/minute for 19 J/cm² with an average ablation depth of approximately 430 μm. The 4 J/cm² is also capable to remove the inner lining of the colon wall, which is evident in figure 4.3 a5 and b5. This capability was also observed at 1030 nm and 4 J/cm² (see section 3.4.2).
Figure 4.3. H&E stained images and 3D profilometric images of ablated tissue using different laser fluences applied at a PRR of 1 kHz and 90% spatial pulse overlap. a1 b1) 19 J/cm² a2 b2) 15 J/cm² a3 b3) 11 J/cm² a4 b4) 8 J/cm² a5 b5) 4 J/cm²
4.3.2 Tissue resection using 10 and 20 kHz PRR at 515 nm

When applying high PRR for tissue resection, the effect of heat build-up or heat accumulation was observed for 1030 nm ablation at 10 kHz and 20 kHz PRR (see section 3.4.2.2.). In high repetition rate operation the heat transfer out of the focal volume is inhibited due to the arrival of successive pulses. Consequently, a heat accumulation effect is generated on the surgical zone. Figure 4.5 shows the 10 kHz ablation area with 90% pulse overlap in the laser scanning direction and laser pulse separation direction. Figure 4.5a shows the 3D profilometric images and figure 4.5b show the histologic results of tissue ablated using the same laser parameters. Maximum thermal damage of 146 µm was observed, which is shown in figure 4.6. The results at 515 nm and 10 kHz PRR with fluences of 19, 15 and 11 J/cm² are similar to the results presented with 1030 nm, 10 kHz laser ablation of tissue using 18, 13 J/cm² (presented in section 3.5.1). From these results, it has been concluded that the high laser fluence (>12 J/cm²) / lower pulse energy resection of porcine tissue in 10 kHz PRR regime was controlled by the effect of plasma generation and thermal effects. The extent of these effects were studied by Vogel et al. using 30 ps laser pulses with 50 µJ pulse energy in distilled water [4.5].
Figure 4.5. H&E stained images and 3D profilometric images of different laser ablated tissues with PRR of 10 kHz, 90% spatial pulse overlap and different laser fluences. a1 b1) 19 J/cm² a2 b2) 15 J/cm² a3 b3) 11 J/cm² a4 b4) 8 J/cm² a5 b5) 4 J/cm²
The lowest fluence used (4 J/cm²) produced an uneven bottom surface to the ablation crater but the edges were smooth and well-defined. These ablations were limited in the mucosa area. It is evident that the thermal damage to lowest laser fluence ablation is very minimal due to the use of the lowest pulse energy to create this surgical zone. However, the profilometric image does show some overall geometrical distortion which is due to the glutaraldehyde fixation process (Figure 4.5 a4 and a5).

Figure 4.6. The maximum depth of thermal damage is marked in the laser ablated area with a laser fluence of 15 J/cm² (47.6 µJ pulse energy) and PRR of 10 kHz. The thermal effects are also present in the bottom surface of the surgical area.

Multiple iterations of the 10 kHz raster scan patterns has been done to examine the capabilities of similar laser fluence to ablate the tissue surface. The ablations were performed for 18, 13 and 5 J/cm² laser fluences with three iterations for each pattern, (shown in figure 4.7). The highest fluences could (18, 13 J/cm²) make deep craters, which was already a realized fact. The lowest fluence of 5 J/cm² also has the capability to create ablation craters using multiple iterations, which is evident in figure 4.7 a3. It is shown that the 10 kHz pattern has the capability to ablate deep craters with higher laser fluences (>12 J/cm²). However, whilst ablation at 10 kHz can alleviate bubble formation the mechanical/thermal damage present does not provide a suitable process for precision laser
surgery. Using a low pulse energy (~14 µJ) can prevent larger areas of thermal damage at the surgical zone. However, the issue when using low pulse energy/low laser fluence is that there is a corresponding reduction in the ablation rate. If these reduced energy parameters are used to remove a 1 mm³ tissue area for example the processing times would be longer. The average depth of the crater in figure 4.7 b3 is approximately 250 µm and single iteration of pattern completed in 25s, so the total time is 75s (three iteration). The projected time to remove a 1 mm³ tissue would be 5 minute.

Figure 4.7 H&E stained images and 3D profilometric images of different laser ablated tissues using a PRR of 10 kHz, 90% spatial pulse overlap and 3 iterations for each patterns with different laser fluences a1 b1) 18 J/cm² a2 b2) 13 J/cm³ a3 b3) 5 J/cm³

For experiments carried out at a PRR of 20 kHz (presented in figure 4.8) tissue ablation occurred due to photodisruption and associated heat effects. The H&E stained images clearly demonstrate the presence of heat affected zones and cavitation/gas bubbles. The mechanical effects play a major role in the 20 kHz PRR tissue resection. During the processing of tissue for H&E staining, some of the thermally damaged tissue areas were removed from the craters. Figure 4.8 b3 clearly demonstrates a deep tissue cut at the edges of the damaged and healthy tissue boundary up to the muscle layer. The presence of this cut was also visible in the corresponding 3D profilometric image (figure 4.8 a3), which
is possibly due to the pressure variation present at the boundary between regions of thermally/mechanically damaged tissue and non-ablated tissue.

Figure 4.8. H&E stained images and 3D profilometric images of different laser ablated tissues using different laser fluences applied at a PRR of 20 kHz and 90% spatial pulse overlap. a1 b1) 19 J/cm² a2 b2) 15 J/cm² a3 b3) 11 J/cm² a4 b4) 8 J/cm² a5 b5) 4 J/cm²
4.3.3. Tissue resection using 40 kHz PRR at 515 nm

Figure 4.9 shows porcine tissue resection using a PRR of 40 kHz. The first three patterns at fluences of 19, 15 and 11 J/cm² clearly show ablation whereas at 8, 5 J/cm² laser fluences a bulge in the tissue sample is created. The 40 kHz pattern completed one iteration in 6.5 second time. Due to the high pulse overlap and short processing time this is likely to initiate the secondary effects of plasma generation and hence the temperature in the tissue will rise due to heat accumulation. Unfortunately, however, there is no associated histology available for these experiments to validate this. The ablation rate of 4.4 mm³/minute was observed with 19 J/cm² ablation of tissue. The rate of ablation graph is presented in figure 4.10. In 40 kHz ablation, the time interval between pulses are very small (25 µs). As discussed previously if the successive pulses do not allow time for the surgical zone to cool, then the heat generated by individual pulses will sum up and create a heat accumulation effect on the tissue. To examine the thermal/mechanical damage, ablation of patterns using 19.5, 18, 17, 16, 15 and 14 J/cm² laser fluences were performed on the tissue surface with a single iteration and very high pulse overlap in the laser scanning direction (97%) and 90% pulse overlap in laser line separation direction, as presented in section 4.5. The results presented with 40 kHz show that the laser ablation with high pulse repetition rate has a combination of plasma induced ablation, secondary effects and accumulated heat. It is proposed that the high pulse overlap and the 40 kHz PRR will cause mechanical disruption to be the dominant factor due to stress and heat accumulation. This is explained in more detail in section 4.5.
Figure 4.9. 3D profilometric images of laser ablated tissue. Different laser fluences were applied at a PRR of 40 kHz and 90% spatial pulse overlap. a1) 19 J/cm$^2$ a2) 15 J/cm$^2$ a3) 11 J/cm$^2$ a4) 8 J/cm$^2$ a5) 4 J/cm$^2$

Figure 4.10 Ablation rate versus laser fluences for 40 kHz PRR and 90% pulse overlap in the laser scanning and laser line separation direction.
4.4. Tissue ablation using different pulse overlap

In this set of experiments, the pulse overlap in the laser scanning direction and laser line separation direction are the same. Figure 4.11. a1 to a8 shows 20 kHz PRR ablation of tissue with 47.6 µJ pulse energy (~15J/cm\(^2\)) and pulse overlap percentage of 94, 92, 90, 88, 86, 84, 82 and 80 respectively. In this experiment, the higher pulse overlap patterns generate a deep surgical zone, which is evident in 94% and 92% overlap pattern operation on the colon. The 94% and 92% overlap patterns with 20 kHz PRR created a deep crater with maximum depth of more than 1mm. For 90% overlap, figure 4.11 a3 shows the same nature of ablation presented in figure 4.8 a2. Both patterns were ablated on tissue with 15 J/ cm\(^2\) laser fluence and 90% pulse overlap. However, it is seen from the profilometric images with 90% pulse overlap in 20 kHz PRR the laser ablated regions have a raised area or bulge in the tissue due to gas bubble formation with the patterns being enlarged after the glutaraldehyde fixation. All the experiments associated with bulges in the tissue after processing show evidence of cavitation/gas bubble formation in the corresponding histology samples (see section 3.4.3, figure 3.20 and section 4.3.2, figure 4.8).

The same laser parameters and pulse overlap were repeated but this time using a PRR of 40 kHz. Here the higher pulse overlap applied (94, 92, 90 and 88%) generated deep craters. The surgical zone shown in figure 4.12 a3, was created with 90% pulse overlap and 15 J/cm\(^2\) laser fluence, which is similar in nature with figure 4.9 a2. It indicates that the ≥90% pulse overlap, >12 J/cm\(^2\) and 40 kHz ablation is capable of producing deep ablation craters, which is also confirmed in the experiments presented in section 4.5 figure 4.17. However, it should be highlighted that although deep craters are produced for the 40 kHz ablation, the histology results in figure 4.17 show that the ablation is not controllable with significant mechanical/thermal damage. Also, it was again observed that a bulge in the laser irradiated tissue occurred < 88% pulse overlap with (15 J/cm\(^2\)) 47.6 µJ pulse energy fluence due to the presence cavitation bubbles.

In conclusion, very high pulse overlap with higher fluence was capable of creating deep surgical zones in 20 and 40 kHz ablation. However, for these parameters significant mechanical/ thermal effects occur within the tissue and hence the ablation is not controllable or desirable for precision laser surgery. To further demonstrate the influence of the mechanical effects for 40 kHz ablation a further set of experiments were carried out with 97% pulse overlap and high laser fluences (section 4.5).
Figure 4.11. 3D profilometric images of laser ablated zones with different laser pulse overlap percentage with 47.6 μJ (15 J/cm²) and 20 kHz PRR. Pulse overlaps are a1) 94%, a2) 92%, a3) 90%, a4) 88% a5) 86% a6) 84% a7)82% a8) 80%
Figure 4.12. 3D profilometric images of laser ablated zones with different laser pulse overlap percentage with 47.6 µJ (15 J/cm²) and 40 kHz PRR. Pulse overlaps are a1) 94%, a2) 92%, a3) 90%, a4) 88% a5) 86% a6) 84% a7) 82% a8) 80%
4.5. Tissue resection using very high pulse overlap (97%) in the laser scanning direction

This set of experiments were performed with 97% pulse overlap in the laser scanning direction and 90% pulse overlap in the laser line separation direction. 1 mm² raster scan patterns were performed for the 3D profilometric measurements and 2 mm² patterns were performed for the histology analysis. The time taken to complete a 1 mm² raster scan pattern for 10, 20 and 40 kHz PRR operations were 84, 42 and 21 seconds respectively. The laser pulse energy was set at 61.32, 57.9, 54.82, 51.05, 47.6 and 43.85 µJ; the corresponding laser fluence was approximately 19.5, 18, 17, 16, 15 and 14 J/cm². The aim of this experiment was to examine the surgical feasibility of high laser fluence with high pulse overlap scanning strategy.

Figure 4.13 shows 10 kHz PRR ablation pattern with high pulse overlap. Using this 97% pulse overlap with high laser fluence, the surgical craters were deep and the tissue resection depth is confined in the mucosa and submucosa region. It has been noticed the maximum thermal damage is up to 150 µm. In this experiment, the pathologist missed the last two patterns during the slicing of the tissue for H&E staining and there are no sections for these parameters. Figure 4.14 is an enlarged image of 4.13 b1. The maximum ablation depth was ~1.5 mm, which is marked in the picture. The damage to the tissue was obvious in the edges of the crater, but that is not in the form of severe thermal and mechanical disruption. The advantage of this high overlap 10 kHz ablation is that there is no evidence of cavitation effects in the resected tissue. The figure 4.13 b4 exhibits geometrical distortions in the surgical zone, which may happen at the time of tissue slicing by the pathologist. The maximum ablation rate achieved in this experiment was 0.7 mm³/minute. This rate of ablation was very low when compared to the 0% and 20% overlap experiments presented later in this chapter. The rate of tissue resection according to the applied laser fluence is presented in figure 4.15.

In conclusion, the 97% pulse overlap in the laser scanning direction and 90% pulse overlap in the laser line separation direction raster scan pattern with 10 kHz PRR and laser fluences between 19.5 and 16 J/cm² produced craters with relatively low thermal damage (~150 µm) compared to the electrocautery and long pulsed laser application in tissue [4.6-7].
Figure 4.13. H&E stained images and 3D profilometric images of different laser ablated tissues using different laser fluences applied at a PRR of 10 kHz and 90% and 97% spatial pulse overlap in laser line separation and laser scanning direction respectively. a1 b1) 19.5 J/cm$^2$ a2 b2) 18 J/cm$^2$ a3 b3) 17 J/cm$^2$ a4 b4) 16 J/cm$^2$ a5) 15 J/cm$^2$ a6) 14 J/cm$^2$
Figure 4.14. H&E stained image of laser ablated tissue using 18 J/cm$^2$ laser fluence. The maximum depth is 1.46 mm and the maximum thermal damage is ~150 µm.

Figure 4.15. Ablation rate versus laser fluences for tissue ablation using 10 kHz PRR, 97% pulse overlap in the laser scanning direction and 90% pulse overlap in laser line separation direction.
The figure 4.16 shows the 3D profilometric images of the high overlap 20 kHz ablation pattern. Unfortunately there is no histology images are available for these patterns. The bottom part of the ablated craters are uneven in shape, which is evident in the profilometric images. Figure 4.17 shows 40 kHz PRR tissue resected area using a high pulse overlap scanning strategy.

Figure 4.16. 3D profilometric images of laser ablated zones with different laser fluences applied at a PRR of 20 kHz. 90% and 97% spatial pulse overlap in laser line separation and laser scanning direction respectively. a1) 19.5 J/cm$^2$ a2) 18 J/cm$^2$ a3) 17 J/cm$^2$ a4) 16 J/cm$^2$ a5) 15 J/cm$^2$ a6) 14 J/cm$^2$
Figure 4.17. H&E stained images and 3D profilometric images of different laser ablated tissues using different laser fluences applied at a PRR of 40 kHz and 90% and 97% spatial pulse overlap in laser line separation and laser scanning direction respectively. a1 b1) 19.5 J/cm² a2 b2) 18 J/cm² a3 b3) 17 J/cm² a4 b4) 16 J/cm² a5b5) 15 J/cm² a6 b6) 14 J/cm²
All the histology images show significant mechanical and thermal damage in the surgical zone. In figure 4.17b3, it is clearly evident that the ablation is deeper at the edges of the patterns where the process stops. The similar although less defined features in the muscle layers are also visible in figure 4.17 b1, b2 and b4. A total time of 84 seconds was taken to complete 40 kHz ablation pattern with 2 mm² area.

A raster scan pattern with 97% overlap in the laser scanning direction and 90% pulse overlap in the laser line separation created a deep (>1 mm) ablated crater using 10 kHz PRR. Here, a maximum thermal damage of ~150 µm and minimal mechanical damage were observed. This is similar to the 10 kHz ablation results presented in section 4.3.2. There is no evidence of cavitation bubbles and the maximum thermal damage is around 146 µm. In the 10 kHz ablation case the cavitation bubble life time is less than the time between individual pulses. The reason is briefly described in section 3.4.3. In 20 kHz ablation, the pattern with 97% pulse overlap created craters, but unfortunately the histology images were not available to assess the effect. At 40 kHz, the 97% pulse overlap raster scan pattern created >1 mm deep craters but with significant thermal and mechanical damage and all the ablation craters reached the muscle layer. In conclusion, the ablation presented with 10 kHz PRR shows a reasonably well-defined ablation crater but thermal damage is not minimal (~150 µm) at high laser fluences and low pulse energy compared to 10 kHz PRR experiments presented in chapter 3. A further drawback is the very low ablation rate and therefore leads to the conclusion that 20 and 40 kHz ablation at 515 nm with high PRR and fluence are not acceptable for precision surgery.

In 2016, Ruby et al. demonstrated the effect of 1 and 33 kHz PRR ablation in bovine bone using a femtosecond laser with high pulse overlap (> 97%) (moving the sample at 1 mm/s) and using approximately 50 µJ pulse energy [4.8]. It was found that at 33 kHz the accumulated heat caused carbonisation and created large fractures at the surgical area. Although in soft tissue ablation the ultrashort pulse ablation dynamics are different to the hard tissue work reported in [4.8], it is an example of how accumulated heat occurs in the high pulse overlap, high pulse energy regime for tissue ablation.
4.6. Colon resection using 0% and 20% pulse overlap

As discussed previously, Dr. Rainer Beck introduced the idea to reduce the pulse overlap in the scanning patterns to improve resection at higher PRR. In Dr Beck’s initial work, the results demonstrated that well-defined craters with low thermal damage regions could be achieved by spacing out pulses spatially using an appropriate scanning strategy. The following work reports experiments conducted to confirm the approach proposed and demonstrated by Dr Beck. To achieve a 0% pulse overlap in 20 kHz PRR, the laser beam was scanned at a speed of 400 mm/s in the laser scanning direction of the outward spiral pattern of 2 mm diameter. The pulse overlap percentage at the spiral line separation direction was set at 90% (spacing of 2 µm). The applied laser fluence was approximately 13 J/cm² (33 µJ pulse energy). In this laser scanning pattern, the laser pulse does not interact with the preceding or successive pulse in the pattern. Therefore the ablation strategy helps to reduce the heat accumulation and accumulated stress effect in the surgical zone. In high PRR and pulse overlap operation, especially at 20 and 40 kHz, the probability of the domination of plasma induced secondary effects in the ablation was very high. Consequently, heat affected and mechanically damaged surgical zones were generated in the tissue. A laser fluence of 13 J/cm² was used to create an efficient surgical effect without cavitation bubbles and thermal or mechanical damage.

The volume of the crater has been calculated using the equation 3.7 presented in section 3.5.3 of chapter 3. The length of the spiral pattern was also calculated using the equation 3.4 presented in section 3.2.5.1 of chapter 3. Here the total length of the spiral pattern was 1570 mm and the total time to scan a 0% pulse overlap pattern was 3.93 second with a speed of 400 mm/s. Therefore, the total processing time for laser pattern iterations of 4, 6, 8 and 10 times takes 15.72, 23.58, 31.44 and 39.3 seconds respectively. The average ablation depth of the surgical zones shown in figure 4.18 a1, a2, a3 and a4 were calculated as 730±110, 812±138, 781±146 and 856±231 µm respectively (the calculation method is presented in section 3.3 of chapter 3). The ablation rates for the 0% pulse overlap pattern are presented in figure 4.19. The maximum ablation rate of 8.74 mm³/minute is achieved for the lowest number of iterations of the spiral pattern. It is evident from the graph that ablation rate is decreasing according to the increase of number of iterations of spiral pattern. The reason behind this process is explained in section 3.5.3 of chapter 3. The ratio of volume of the crater to total time to complete the pattern is considered as the ablation rate of this surgical process. The histology images show well-defined ablation patterns with minimal thermal damage. A thermal damage zone less than 60 µm was
observed. However, the mucosal area in the samples shown in figure 4.18 b3 and b4 became damaged or were removed during the processing of tissue for histology. Also, in these figures the muscle tissue is showing significant tearing not seen in previous histology samples but is attributed to the sample preparation for histology and not an effect of the laser processing.

Figure 4.18. H&E stained images and corresponding 3D profilometric images of laser ablated zones with 13J/cm² laser fluence, 20 kHz PRR, 0% pulse overlap in direction of laser scanning and a different number of iterations of scanning patterns. a1 b1) 4 times, a2 b2) 6 times, a3 b3) 8 times and a4 b4) 10 times.
Figure 4.19. Ablation rate versus number of iterations of the spiral scan pattern using 13 J/cm² at 20 kHz PRR and 0% pulse overlap in the laser scanning direction.

As a comparison a 20% pulse overlap in the scanning direction was investigated by setting the galvo scanning speed at 320 mm/s for 20 kHz. The number of iterations were set to four for the 20% pulse overlap ablation.

The 20% overlap ablation produced a crater with well-defined edges which is evident in figure 4.20 a1 and b1. The achieved ablation rate was 7.75 mm³/minute, which is less than the ablation rate achieved in 0% pulse overlap (with 4 iterations and a PRR of 20 kHz). The 0% pulse overlap strategy is more efficient than 20% pulse overlap ablation with the main difference being the time taken to complete the pattern, which for 0% overlap ablation is 3.93 s compared to 4.9 s for a 20% overlap pattern. The figure 4.20 a2 shows the 40 kHz ablation of 0% pulse overlap (number of iterations 4). To achieve a 0% pulse overlap the galvo mirrors scanned at a speed of 800 mm/s. The average depth of ablation was 743±120 µm, achieved in 7.84 seconds processing time. Here the ablation rate is very high (17.85 mm³/minute), which is almost double the maximum ablation rate achieved using 0% pulse overlap in 20 kHz PRR of operation. Also, at 40 kHz the surgical area is not showing any evidence of significant thermal damage. These results again clearly show the benefit of using 0% pulse overlap enabling a higher PRR to be used and increasing overall ablation rates.
Figure 4.20. H&E stained images and corresponding 3D profilometric images of laser ablated zones with 13 J/cm$^2$ laser fluence, pulse repetition rate of 20 kHz (a1, b1), 40 kHz (a2, b2), pulse overlap of 20% (a1, b1) and 0% (a2, b2) in direction of laser scanning. The number of iteration for laser scanning pattern was set at 4.

4.7. Conclusions

The main potential advantage of using 515 nm is the application of high laser fluences/lower pulse energy for tissue ablation compared to the results presented in chapter 3 at 1030 nm. This was possible because of the smaller focused spot size of 515 nm laser which allowed for higher fluences incident on the tissue even though the maximum average power for this laser at 515 nm is lower than that for 1030 nm. It should also be noted that, whilst not investigated in this thesis, picosecond laser transmission at 515 nm through a hollow core negative curvature fibre has previously been demonstrated by [4.9].

The tissue resection using a wavelength of 515 nm, pulse width of 6 picoseconds and PRR of 1 kHz was presented in the first part of this chapter. The selection of applied pulse energy/laser fluence is an important factor for tissue resection. In this PRR regime the highest fluence of 19 J/cm$^2$ to lowest fluence of 4 J/cm$^2$ were used for the ablation. It has been noticed that the ablation depth varies according to the applied laser fluence. The
maximum necrotic tissue width of 80 µm has been presented with 19 J/cm² laser fluence ablation. The ablation rate of this ablation regime was found to be relatively low, approximately 0.1 mm³/minute, for the maximum average ablation depth achieved.

The 10 kHz ablation demonstrated ablation craters in high laser fluence operation but with an increased maximum thermal damage of approximately 146 µm (compared to the 1 kHz operation). This damage was due to combined secondary effects of plasma generation and heat accumulation due to relatively high PRR, which is evident in histology images. However, the histology image shows negligible thermal damage in 10 kHz ablation using lower laser fluence of 4 J/cm² although the crater depth was reduced (<100 µm). Multiple iterations of 10 kHz raster scan patterns were also presented. It has been observed that the lowest fluence operation of 5 J/cm² has the capability to produce deep craters in the order of 300 µm when multiple iterations (3) of the scanning pattern is applied to the tissue surface.

The 20 kHz ablation with 90% pulse overlap was a clear example of secondary effects of plasma generation and the heat accumulation due to high PRR. Evidence of cavitation was observed in the histology samples. For a PRR of 40 kHz with a 90% pulse overlap the 3D profilometric images shows a deep craters of > 500 µm length for higher laser fluences (>12 J/cm²) but unfortunately no histology images are available to reach a conclusion. The histology results presented in section 4.5 (with a high pulse overlap (97%) using 40 kHz) shows high mechanical and thermal damage in the tissue.

The tissue ablation using different pulse overlap with a constant laser fluence (15 J/cm²) show that the higher pulse overlap (>90%) create deep (>1 mm) craters. It has been noticed that the change in pulse overlap in the laser line separation direction was not a good choice for performing tissue ablation. In light of this experiment, the next set of experiments performed with 97% pulse overlap and 90% pulse overlap in the laser scanning and laser line separation direction respectively. Different laser fluences applied on the tissue surface ranging from 19.5 to 14 J/cm². In 10 kHz PRR, the histology images show a precise size and shaped surgical zone with a maximum thermal damage of 150 µm and an ablation rate of 0.7 mm³/minute. On the other hand, the 40 kHz ablation also produced deep craters but significant mechanical/thermal damage of the craters was clearly visible in the H&E stained images. In most of the surgical area, the concentration and accumulation of energy (due to high PRR) deposition causes overheating and thermal stress. The 10 kHz PRR ablation with higher laser fluences and high pulse overlap created thermal damage in the surgical zone which is still better in comparison with electrocautery.
and other conventional tools. However, using 20 and 40 kHz PRR with high laser fluence/pulse energy is not a good choice for procedures such as colonic tissue surgery, where thermal damage matters.

To reduce the total processing time and alleviate secondary effects of plasma formation and heat accumulation in the tissue surgery, the laser pulses were separated to 0% pulse overlap (no overlap) with 20 kHz PRR. In this PRR regime, the maximum ablation rate achieved was 8.74 mm³/minute (for 4 iterations) with deep (>1 mm) and well-defined surgical zones. The 20% pulse overlap and 4 pattern iterations were also performed to compare with the 0% overlap ablation strategy. The ablation rate achieved in 20% pulse overlap was less than the ablation rate observed in 0% pulse overlap ablation.

The 0% pulse overlap pattern was also performed on the tissue surface using 40 kHz PRR with 4 iterations to examine the ablation depth and thermal damage. A well-defined resected zone was observed. It should be highlighted that to achieve a 0% pulse overlap for a 40 kHz PRR provides a significant practical challenge because the optical fibre (during endoscopic application) would need to be scanned in way which allows speeds of the focused spot on the tissue surface of 800 mm/s (for a focused spot size of around 20 µm). However, the scan speed could be reduced (to achieve 0% overlap) by reducing the focused spot size of the laser beam. A reduced focal spot size may result in an increased duration of tissue surgery but not significantly (in the optimal ablation strategy an average depth of 743±120 µm is achieved in 7.84 seconds). These results therefore provide strong evidence that this surgical modality would be capable of resecting early stage lesions/polyps in the colon supported by histology.

4.8. References


515 nm picosecond laser resection of cancerous and non-cancerous lung tissue: A pilot study in an ovine model

5.1. Introduction

There have been several studies on the effect of ultrashort pulse lasers in biological tissues [5.1-4] as discussed previously. Building on that, the study reported in this chapter looks to examine the effect of ultrafast visible laser on Ovine Pulmonary Adenocarcinoma (OPA) affected lung tissue and healthy lung tissue of a sheep. OPA is a type of lung cancer affecting the pulmonary neoplasia of a sheep as a result of infection from the Jaagsiekte Sheep Retrovirus (JSRV) [5.5]. Ablation characteristics of this laser need to be analysed to identify the opportunity of picosecond visible laser in lung surgery. Whilst it is not proposed that laser surgery would be used to treat JSRV, sheep which have the disease identified are culled, ovine lung tissue could act as a model for human lung cancer studies [5.6]. Lung Volume Reduction Surgery (LVRS) is a treatment modality to remove the least functional areas of lungs in order to improve the breathing condition of patients having Chronic Obstructive Pulmonary Disease (COPD) [5.7]. Laser ablation on lung tissue has been performed by different research groups but the focus was on using lasers of continuous wave and long pulse duration [5.8-10]. In 2018, Joseph et al. performed an Nd: YAG laser resection of pulmonary metastasectomy on a group of patients using a 600 µm core optical fibre for the delivery of pulses [5.10]. Recently, similar surgeries were reported [5.11-12] with the laser resection being carried out at the metastasis stage (outside the scope of our application for high precision surgery). The pulse duration used for this applications varied from 0.1 s to 10 s.

The effectiveness of picosecond green laser in lung surgery is also examined in this exvivo study. The main advantages of picosecond green laser ablation are surgical accuracy due to small laser spot diameter (20 µm) and less thermal damage (<100 µm). OPA has several similarities with human lung cancer; a detailed description of the OPA model can be found in [5.13-14]. In this case the tissue used was from natural cases of clinical OPA. Only initial feasibility trials were carried out here in order to assess the viability of the picosecond laser for ablation of this different tissue type. The experimental setup is same as described in section 4.2 of chapter 4. All experiments on the lung tissue were carried out with the assistance and guidance of Dr Rainer Beck.
5.2. Healthy and cancerous lung tissue ablation using 5 and 10 kHz PRR USPs

The laser ablation of lung tissue was carried out on healthy and cancerous samples. On each sample, five separate areas were ablated with the same scanning pattern but with different laser pulse energies. A 1mm$^2$ raster scanned area was ablated using 5 kHz and 10 kHz PRRs. The laser line spacing was set at 2 µm to achieve 90% pulse overlap in the laser line direction of the raster scan pattern for a focused laser spot diameter of 20 µm.

The scanning speed was varied according to the PRR to achieve 90% pulse overlap. The laser pulse energy was set at 58.57, 47.6, 36, 24.17 and 13.27 µJ; the corresponding laser fluences were approximately 19, 15, 11, 8 and 4 J/cm$^2$. The aim of using these fluence changes was to examine the effect on ablation depth and the width of damage. Also, the use of a 20 kHz PRR for ablation of OPA affected lung tissue is presented in this chapter with a laser fluence of 15 J/cm$^2$ and approximately 70 and 90% pulse overlap in the laser scanning direction and laser line separation direction respectively.

Examples of surface profiles of an ultrafast laser resected tissue are shown in figures 5.1 and 5.2. The maximum depth of ablation in the healthy tissue is achieved using the maximum pulse energy applied (which is 58.5µJ). However, a significant difference in the ablation depth is noted for cancerous and non-cancerous lung tissue. In cancerous tissue the depth of ablation is less than half when compared to the ablation depth of healthy tissue as seen in figures 5.1 and 5.2. The figures on the left show laser ablated healthy tissue and on the right show laser ablated cancerous tissue. For a PRR of 5 kHz the maximum average ablation depth achieved in healthy tissue using the parameters shown in figure 5.1 was 704 ± 321 µm whereas for cancerous tissue the maximum achieved ablation depth was 363 ± 86 µm. The error bar represents the variation in the resection depth measurement from different regions of the ablation crater. For a PRR of 10 kHz the maximum average ablation depth achieved in healthy tissue using the parameters shown in figure 5.2 was 742 ± 388 µm whereas for cancerous tissue the maximum achieved ablation depth was 286 ± 38 µm.

Figures 5.3 and 5.4 shows a graph revealing the importance of laser fluence as a function of crater depth in tissue surgery. Figure 5.3 represents the average ablation depth of healthy and OPA affected sheep lung tissue over a range of laser fluences at a PRR of 5 kHz (corresponds to figure 5.1). Figure 5.4 represents the average ablation depth of healthy and cancerous lung tissue with same laser parameters except using a PRR of 10 kHz (corresponds to figure 5.2). There is a general trend for the depth of ablation to
increase according to an increase in the laser fluence. However, there are some anomalous results where the ablation depth decreases with increase fluence. The reason behind this is due to the change in the laser focus because of the highly uneven structure of the tissue surface, as can be seen in figure 5.1 and 5.2.

Figure 5.1. 3D profilometric images of laser ablated healthy (pictures on left side) and cancerous (pictures on right side) tissue. Different laser fluences were applied at a PRR of 5 kHz and 90% spatial pulse overlap. a1b1) 19 J/cm² a2b2) 15 J/cm² a3b3) 11 J/cm² a4b4) 8 J/cm² a5b5) 4 J/cm²
Figure 5.2. 3D profilometric images of laser ablated healthy (pictures on left side) and cancerous (pictures on right side) tissue. Different laser fluences were applied at a PRR of 10 kHz and 90% spatial pulse overlap. a1b1) 19 J/cm² a2b2) 15 J/cm² a3b3) 11 J/cm² a4b4) 8 J/cm² a5b5) 4 J/cm²
It is clear that the ablation depths in cancerous tissue are lower than for healthy tissue for a given fluence. The morphological difference of cancerous tissue is the fundamental reason behind this issue which is discussed further below. Nevertheless these initial results demonstrate the feasibility of an ultrashort pulsed laser surgical modality with control of the ablation depth by varying the pulse parameters and scan parameters associated. Thus the deployment of ultrashort laser surgery could be effective where avoidance of lung tissue damage is critical.

Figure 5.3. Comparison of healthy and cancerous tissue ablation depth achieved using 6 picosecond laser. Average ablation depth versus laser fluences for 5 kHz PRR and 90% pulse overlap in the laser scanning and laser line separation direction.

Figure 5.4. Comparison of healthy and cancerous tissue ablation depth achieved using 6 picosecond laser. Average ablation depth versus laser fluences for 10 kHz PRR and 90% pulse overlap in the laser scanning and laser line separation direction.
The ovine lung tissue affected by OPA exhibits a higher density in comparison to the healthy tissue and as a consequence a significantly reduced tissue removal rate can be expected compared to healthy lung tissue. Figure 5.5, depicts the tissue density change of an OPA affected tissue and healthy lung tissue in a sheep. Two distinct circular shaped areas can clearly be seen which represent the OPA affected area. Healthy tissue surrounds these areas. It can be seen that the healthy hollow alveolar tissue has changed to thick cuboidal or columnar cells as result of the lung cancer. At the final stage of OPA affected lungs the cancerous tissue is very dense and up to three times heavier than healthy lung [5,15]. Nevertheless, a set of parameters were found to remove and ablate the tissue by means of picosecond laser pulses at a wavelength of 515 nm and a raster scanning approach.

![Figure 5.5. Healthy and OPA affected parts in a sheep lung tissue. The segregated cancer tissue (circular shaped shaped) inside a healthy lung is shown in the picture][5.15]
Figure 5.6. Healthy lung histology

Figure 5.7. H&E stained images of healthy (a1, a2) and cancerous (b1, b2) sheep lung tissue. 5 kHz PRR, 90% pulse overlap and different laser fluences of a1, b1) 19 and a2, b2) 8 J/cm²
The lung is composed of thin walled alveoli surrounded by squamous epithelium, shown in figure 5.6. The collagen and elastin are the basic building blocks of alveoli. The alveoli loses its elasticity as a result of adenocarcinoma. The healthy lung tissue consists of alveoli, bronchiole, blood vessels, cartilage, smooth muscle layers and connective tissues. In 5 and 10 kHz PRR ablation of healthy and cancerous tissue, the available histology results are presented in figure 5.7 and 5.8. From the histology samples it is evident that the laser ablation created deeper craters in healthy lung tissue when compared to the ablation depth in cancerous tissue. In figure 5.7 a2 and 5.8 a2, the laser pulses incident on the edges of a bronchiole, which is surrounded by smooth muscle layers and connective tissue. In 5 and 10 kHz ablation of tissue, the region of thermal damage has a thickness of around 50 μm. However, in figure 5.7b2, the thermal damage is larger than 50 μm.
In healthy tissue ablation, the squamous epithelium of the alveoli and the connective tissue are intact beyond the ablated cavity region. However, the non-uniform structure of the lung can have a significant impact on the uniformity of the laser resected cavity as shown in the histology section (figure 5.7a1 and 5.8a1). The depth of the laser ablation can be regulated by the applied pulse energy (see figure 5.8). For early stage cancer, this surgical modality could be a suitable approach to resect the neoplasm by laser ablation with minimal thermal damage to the surrounding tissue.

The interaction of the ultrafast laser to the tissue mainly depends on the morphology of the tissue. Consequently, the dynamic behavior of the plasma mediated ablation process can be modulated by both elasticity and strength of the tissue [5.16] and therefore tumors have a different response to healthy tissue. For example, the stiffness of a prostate tissue can be used as a biomarker for prostate cancer [5.17] and strain elastography is identified as a tool for differentiating adenoma and adenocarcinoma in rectum [5.18]. These studies are examples that highlight the change in tissue stiffness of cancerous and healthy tissue. Nevertheless, this early evidence shows that there is a measureable difference between the picosecond laser ablation of healthy tissue compared with cancerous tissue provides potential to develop a selective ablation.

The tissue stiffness is defined as the resistance to deformation or cut induced by external forces. In 2008, Hoyt et al. investigated the stiffness of cancerous and healthy prostate tissue of human [5.19]. They found that the cancerous tissue is significantly stiffer than healthy tissue by a factor around 2 to 2.5. This result indicates that the tissue stiffness in cancerous prostate tissue varies from 7.8±3.3 to 40.6±15.9 kPa and in healthy tissue stiffness varies from 3.8±1.8 to 16±5.7 kPa. In comparison, the cancerous prostate tissue stiffness value is more than double compared to healthy prostate tissue. The stiffness of the healthy and cancerous human breast was measured by Plodinec et al. for the diagnosis of breast cancer [5.20]. They found that the stiffness in healthy breast varies from 1.13±0.78 to 1.83±1.13 kPa and in cancerous breast it varies from 1.91±0.99 to 3.68±1.92 kPa. The variation of stiffness value in both tissue is in the range of 1.7 to 2. The above results clearly indicate that the cancerous lung tissue may exhibit more resistance to the incident laser beam compared to the healthy lung tissue. This can be a reason for the average ablation depth difference in cancerous and healthy tissue. More morphological analysis of cancerous and healthy tissue needs to be done to reach a final conclusion, which is beyond the scope of this work.
5.3. Cancerous lung tissue ablation using 20 kHz PRR USPs

Figure 5.9. 3D profilometric (a1) and H&E stained (b1) images of cancerous sheep lung tissue ablated using 20 kHz PRR, 90% pulse overlap in laser line separation direction and 70% overlap in laser scanning direction and laser fluences of 15 J/cm²

Figure 5.9 shows the laser ablated cancerous tissue using 15 J/cm² applied laser fluence and approximately 70% pulse overlap in the laser scanning direction and 90% pulse overlap in the laser line separation direction. The depth achieved in both samples (the glutaraldehyde fixed and formalin fixed) are similar. The ultrashort pulse laser ablation of lung tissue is an unexplored area of surgery, where the zero damage to healthy lung tissue is paramount. It has been observed that cavitation bubbles (gas bubbles) and shock wave induced mechanical damage occurs when porcine colon was ablated with 20 kHz PRR and very high pulse overlap in both the laser scanning direction and laser line separation direction (as discussed in detail in section 4.3.2). To alleviate this then the pulse overlap must be reduced. However, the ablation of 0% and 20% pulse overlap in scanning direction for lung tissue was not performed here as there was limited tissue availability (as discussed in proposed further work).

5.4. Conclusions

The picosecond laser was operated at a wavelength of 515 nm and 6 ps to resect square cavities from fresh ex-vivo OPA samples and healthy lung samples using a range of scanning strategies. An efficient ablation of cancerous and healthy lung tissue with minimal thermal damage is presented. The ablation depth can be regulated by means of the laser pulse energy. The laser ablation in healthy pig tissue and healthy porcine tissue are different because of the morphological changes in both tissues. In cancerous phase, the sheep lung tissue loses its elasticity and transform to a densely packed smooth
columnar cells that has more resistance to the laser pulses, which is evident in the experimental results. The cancerous tissue exhibit low ablation rate compared to the healthy samples with same laser parameters. In 5 kHz PRR laser ablation of cancerous tissue, the maximum and minimum depth achieved (using maximum and minimum fluence) was 363±86 µm and 98±20 µm respectively. These ablation depths are almost half of the ablation depth achieved in healthy lung tissue. The H&E stained image of 20 kHz PRR ablation of 70 % pulse overlap shows a maximum depth approximately 500 µm, which is a good sign for the ablation of early stage cancer. In conclusion, the pilot study presented in this chapter demonstrates the potential of ultrashort lasers at 515 nm for high precision laser resection of ovine lung tissue.

5.5. References


Chapter 6

Hollow Core Negative Curvature Fibre delivered ultrashort laser pulse characterisation and application in laser colon tissue resection

6.1. Light Guidance mechanism of Hollow Core Negative Curvature Fibre (HC-NCF)

The light guidance mechanism of HC-NCF can be described by a 2 dimensional (2D) antiresonant reflecting optical waveguide (ARROW) model [6.1]. The figure 6.1 illustrates the principle of ARROW guiding mechanism which shows a low index core surrounded by two high and low index layers of cladding. In this model, the higher index core wall (cladding) acts as a Fabri Perot (F-P) resonator. The important aspect of the ARROW waveguide is the F-P antiresonant reflectors are used for the light confinement in the core. The F-P cavity reflects back all the wavelengths which do not match the resonant wavelength of the cavity and hence they propagate through the low index core. These antiresonant wavelengths propagate through the core with low loss because of destructive interference in the F-P cavity. On the other hand, the wavelengths of light which match the resonant wavelength of the F-P cavity leak out from the core.

The anti-resonant model can be expressed by [6.2],

$$\lambda_{Anti} = \frac{4d}{2m+1}\sqrt{n_2^2 - n_1^2} \quad m=0, 1, 2 \text{ etc.} \quad (6.1)$$

where ‘d’ is the thickness of the cladding layer, $n_1$ and $n_2$ are the refractive indices of the core and cladding respectively. The ARROW model is valid only when $d/\lambda >> 1$. 

Figure 6.1. Two dimensional representation of the ARROW structure (top) and corresponding transmission spectrum (bottom) [6.1].

The laser pulse delivery through a standard single mode fibre is based on total internal reflection (TIR) between the solid silica core and cladding. When transmitting ultra-short pulses (USPs) through the solid core fibres, pulse distortions occur as a consequence of the non-linear effects generated in the fibre such as self-phase modulation and self-focusing [6.3-4]. Conversely, the proposed HC-NCF guides the laser light through the air core based on anti-resonant reflecting optical waveguide (ARROW) technique [6.5] described in figure 6.1. This minimizes the nonlinear effects in the fibre due to high peak power pulse delivery, making the HC-NCF an attractive tool to deliver USPs. However, the optimization of the maximum power delivered through the HC-NCF is challenging due to the low numerical aperture and relatively small core size of the fibre (< 22 µm). If not correctly aligned, the high peak power pulses create thermal or ionization damage at the light coupling end face of the fibre (Damage threshold of silica is 4 J/cm² [6.6]). The highest coupling efficiency determines the amount of maximum power delivered through the fibre and the damage threshold power of the fibre. A poor alignment (poor coupling efficiency) can lead to the damage of the fibre before its maximum power handling capacity is reached.
6.1.1. Factors affecting coupling efficiency of seven cell HC-NCF

The seven cell HC-NCF used in this work was developed using stack-and-draw technique. The main factors to consider when optimising the coupling efficiency are:

- Numerical Aperture (NA) of the fibre and coupling lens
- Mode field diameter of the fibre
- Focal length of the coupling lens
- Laser beam diameter incident on the coupling lens
- Quality of the fibre end face cleave

The light gathering capacity of the fibre is known as the numerical aperture (NA). The light guidance through a standard fibre is based on total internal reflection (TIR). The numerical aperture is described as the $\sin \theta$ of the maximum angle created by an incident ray of light from air to the fibre core, which satisfies the guidance of light through the fibre core.

$$NA = n \sin \theta$$ (6.2)

Where $n$ is the refractive index of the medium outside the core and $\theta$ is the acceptance angle. Figure 6.2 shows the change in focused laser spot diameter and NA of a lens for different focal length lenses for a given input beam diameter on the lens of 2.59 mm (measured beam diameter of Carbide laser). The measured NA of the seven cell HC-NCF is 0.044 (measured by Dr. Richard Carter) with a physical core diameter of 21 µm and the mode field diameter (MFD) is approximately 15 µm shown in figure 6.3.

![Figure 6.2](image)

Figure 6.2. A graph showing the change of NA and focused spot size with focal length of the lens for a given input beam diameter on the lens of 2.59 mm. The crossover point in the graph is the point where the NA of the lens matches the NA of the fibre with an optimized focused spot size and focal length of the coupling lens.
Figure 6.3. Mode field profile of 1030 nm laser delivered through seven cell NCF (Top). Associated transverse profile through the centre of the mode field (bottom).

For an efficient coupling the numerical aperture of the coupling lens should be equal to or less than the numerical aperture of the fibre. The numerical aperture of the lens can be calculated by,

$$NA = \frac{D}{2f}$$  \hspace{1cm} (6.3)

Where $D$ is the diameter of the beam incident on the coupling lens and $f$ is the focal length of the lens. In the experiment, the $1/e^2$ diameter of the laser beam was 2.59 mm and the laser beam quality <1.3. A plano-convex lens with 29 mm focal length gives an exact match with the NA of the fibre (for an input beam on the lens of 2.59 mm).

Due to availability, a 30 mm focal length planoconvex lens was used to focus the laser beam to the laser launching face of the fibre. Figure 6.4 shows the schematic representation of laser focusing using a lens. The estimated diameter of the laser focal
spot was ~ 19 µm with a numerical aperture value of the lens calculated as 0.0431 using equation 6.3,

\[ 2W_0 = \frac{4 \lambda f M^2}{\pi D} \]  \hspace{1cm} (6.4)

\( \lambda \) is the wavelength of the laser, \( f \) is the focal length of the coupling lens, \( D \) is the diameter of the laser beam incident on the coupling lens and \( M^2 \) is the laser beam quality and \( W_0 \) is the radius of the beam at the waist.

The relation between NA of the lens, focal length of the lens and the focused spot diameter can be characterised by using equations 6.3 and 6.4.

Another important factor affecting the coupling efficiency is the quality of the fibre end faces. The cleave must not damage the microstructured cladding and provide a clean, planar finish. Figure 6.5 shows the quality of the fibre cleaving achieved with the ceramic blade which was deemed acceptable for fibre coupling experiments. Based on practical
experience the ceramic blade provides a better cleave compared with other commercially available fibre optic cleavers

6.2. High peak power laser pulse delivery through HC-NCF

The ultrafast laser delivery through seven cell HC-NCF was performed for 1.5 m of fibre. The laser beam from the tunable pulse width laser (CARBIDE, LIGHT Conversion) focused to the fibre core through the planoconvex lens of focal length 30 mm. The spot size at the focal point was \( \sim 19 \, \mu m \) \((1/e^2)\) and the NA of the lens was 0.04321, which is approximately equal to the NA of the fibre (0.044). The fibre had an MFD of \( \sim 15 \, \mu m \) and hence there was a mismatch in the focused spot size diameter and the MFD of the fibre which resulted in slightly reduced coupling efficiency. However, a maximum coupling efficiency of 70% was achieved. Initially the laser to fibre alignment was conducted with very low power to avoid the accidental damage of the cladding of HC-NCF. The rest of the 30% power with lower energy attenuated while propagating through the fibre. During the initial alignment process the proximal end of the fibre is connected to a fibre connector. The images of the output end face of the fibre was achieved using a 20X objective and a BASLER USB camera. The fibre output end face image is shown in figure 6.6. This method enabled the position of the fundamental mode in the fibre to be checked, which is shown in figure 6.6 a and 6.6 b. A strong fundamental mode was achieved in the fibre core by adjusting the multi-axis stage at the input coupling face of the fibre. After the alignment, the objective lens and camera were replaced with a power meter (OPHIR) which continuously monitored the output power. If any increase in the output power was observed while further adjusting the multi-axis stage, then the imaging experiment was repeated until the fundamental mode was confirmed to be confined in the core with a maximum power delivered at the fibre output.

![Figure 6.6](image-url)

Figure 6.6. a) Near field image of single mode light guidance pattern through the hollow core fibre b) image of a mode field pattern at the fibre end face.
Figure 6.7 shows the coupling efficiency of different pulses having pulse widths ranging from 8 ps to 232 fs. The test was performed at 60 kHz with 2 W average power and hence the maximum pulse energy from the laser was 33 µJ. It is evident from the graph, from 8 ps to 2 ps the HC-NCF could deliver approximately 70% of the power from the laser including the fibre loss of 0.19 dB/m at 1028 nm. On the other hand, from 232 fs to 1ps there is a gradual decrease in the measured output power. As discussed in detail in section 6.2.1, this is due to the non-linear effects in the fibre at shorter pulse widths. The non-linear effects in the fibre include self-phase modulation, self-focusing and soliton formation. This shifts the energy into wavelengths beyond the range of the detector (wavelength range: 0.8 to 3 µm) and hence a drop in output power is measured. The strength of this shift increases with increasing power hence the lower output power is measured at higher input powers. In the lower pulse width regime the HC-NCF generated additional wavelengths which is evident from the spectral measurements.

![Figure 6.7. Input to output power coupling plot of 1028 nm laser to seven cell HC-NCF with different pulse widths at 60 kHz PRR and 2 W maximum output regime.](image)

Figure 6.8 shows the output versus input pulse energy plot for the seven cell HC-NCF. The point representing a 70% efficiency are also plotted for comparison. Up to the input pulse energy of approximately 42 µJ and peak power of 7 MW the measured values follow the 70% slope. The pulse energy at the fibre output gradually reduces with a further increase of input pulse energy. The gradual decrease of output pulse energy was due to the onset of damage into the cladding structure around the hollow core. At an input pulse energy of 71 µJ and peak power of 11.8 MW the output power decreased to zero due to
total destruction of the core and cladding structure as shown in figure 6.9 a. This damage is due to the spatial periphery part of the laser pulse reaches the maximum fluence to destroy the silica cladding completely, shown in figure 6.9 b.

Figure 6.8 Input to output pulse energy plot of 7 cell HC-NCF with a wavelength of 1028 nm, pulse width of 6 ps and 60 kHz PRR at 5W maximum output regime.

Figure 6.9. a) Damaged input facet of HC-NCF showing complete destruction of the core and cladding structure. b) Effect of spatial periphery part of laser pulse in cladding destruction.
6.2.1. Spectral and temporal quality measurement of laser and fibre delivered laser pulses

The fibre delivered pulses were characterised by using an autocorrelator (APE PulseCheck) and spectrometer (Ocean optics). The temporal and spectral quality of the output pulses were measured with respect to the input pulses. Figure 6.10 shows the experimental setups for the measurement of pulse width and the spectral output of the fibre delivered pulses. A combination of a half wave plate and a beam splitter were used to control the optical power to the autocorrelator and spectrometer to ensure power was kept below the maximum power handling capacity of those devices. Autocorrelator measurements were made on the direct laser beam and the laser beam through 1.5 m of HC-NCF, shown in figure 6.10a, to assess the temporal distortion of laser pulses delivered through the fibre. It was observed that the 6 ps pulse duration is well maintained after the laser pulse is transmitted through a 1.5 m length of HC-NCF as shown in figure 6.11. This result clearly indicates that no pulse dispersion occurs in the output pulse with ~29 µJ pulse energy. The spectral characteristics of the laser and the fibre delivered beam are shown in figure 6.12. The laser pulses delivered through HC-NCF are well preserved with no additional wavelengths apparent in the spectrum due to non-linear effects such as soliton formation, self-focusing or Raman scattering [6.7-9]. However, a slight shift in the centre wavelength of the delivered pulse in comparison to the laser is noted. This is due to the non-uniform attenuation of the HC-NCF at the wavelength of interest (measured). The attenuation spectrum in this wavelength region of seven cell HC-NCF is shown in figure 6.13 (data provided by Dr. Fei Yu, University of Bath). When the values of attenuation in the wavelength of interest are applied to the spectrum from the laser, the same spectral shift is noted (as observed in the measured delivered pulse). This, attenuation modified, waveform is shown in figure 6.14 and matches well to that of the delivered pulse shown in figure 6.12. It was found that for pulse widths above 2 ps the temporal and spectral quality of the pulse was preserved after propagation through 1.5 m of HC-NCF. However, for pulse widths below 2 ps, a change in the spectral and temporal shape of the fibre delivered pulse was measured.
Figure 6.10. Experimental setup to measure a) the pulse width of the fibre delivered pulse b) the spectrum of the fibre delivered pulse

Figure 6.11. Autocorrelation traces of picosecond laser beam (FWHM = 6ps) with ~41 µJ pulse energy from the laser and ~29 µJ pulse energy from HC-NCF delivered pulse
Figure 6.12. Optical spectra of laser beam of 6 ps pulse width and 41 µJ pulse energy. 1.5 meter long HC-NCF delivered beam with 6 ps pulse width and ~29 µJ pulse energy (Blue line).

Figure 6.13. Attenuation spectrum of 7 cell HC-NCF

Figure 6.14. The 6ps laser pulse from the fibre (red line) and another pulse generated using the attenuation data of corresponding wavelength (blue line).
The spectrum of the fibre delivered pulses was measured with an input pulse energy of 41 µJ and a pulse width of 600 fs. The actual pulse width is 0.65 times the width of autocorrelation traces. The spectrum has the form of supercontinuum/wide band laser and the observed laser emission spectrum through the fibre is shown in figure 6.15. A spectral range of visible light is observed through the fibre cladding when sub-ps laser pulses propagate through the fibre indicative of the non-linear process and can be seen clearly in figure 6.17. Additionally, the measured pulse width of the fibre delivered pulse was 1 ps at this transmission condition showing significant pulse stretching (from the input
pulse width of 600 fs) shown in figure 6.16. In 2017, Yatsenko et al. demonstrated the generation of a supercontinuum laser ranging from 400 nm to 1600 nm in a hollow core fibre with light propagation based on ARROW technique [6.10]. This was generated using a laser with a wavelength of 1028 nm, pulse width of 250 fs, pulse repetition rate of 1 kHz and a maximum input pulse energy of 130 µJ.

Figure 6.17. Spectral range of light (“rainbow”) observed along the length of the fibre due to non-linear effects for a 1.5 m long piece of HC-NCF. The input pulse energy is 41 µJ and the input pulse duration is 600 fs.

6.3. Practical implementation of tissue resection using fibre delivered pulses

Ultrafast lasers are a valuable tool to realise procedures with exceptional precision and minimal thermal damage in biological tissue resection [6.11-12]. This capability makes fibre delivered ultrashort pulse lasers a promising surgical device for minimally invasive colonic surgery. The surgical procedure in endoluminal gastroenterology requires high precision and minimal necrotic tissue because bowel perforation is generally identified as one of the most severe complications after colon surgery. However, until recently there were no suitable flexible fibre delivery methods for ultrashort laser pulses with high peak
powers preventing the deployment of ultrashort lasers in new, minimally invasive or endoscopic procedures. The development of novel hollow core microstructured fibres has enabled the potential for delivery of such pulses throughout the body.

Therefore, in this section, a study on the ultrashort laser pulses suitable for precision porcine colon resection flexibly delivered via a hollow core negative curvature fibre (HC-NCF) is reported. During the laser ablation the fibre is manipulated via multi-axis robotic device to mimic a more practical arrangement that might be expected during a surgical procedure in an operating theatre. In a practical theatre the laser would need to be located remotely from the patient on the operating table. The fibre would then need to allow an arbitrary path to be followed around the theater (likely to be a few meters from the laser to the endoscopic device) to avoid other pieces of equipment and not get in the way of the medical staff. This fibre would then be guided down the endoscope to the surgical site (in the colon for example). The fibre end would then be manipulated or repeatedly scanned (rastered) over small distances (in the order of a few mm) during the surgical procedure. The experimental set-up (as shown in figure 6.18) replicates this practical arrangement.

The HC-NCF based laser resection of tissue was performed on a porcine colon tissue as a proof of concept using 1028 nm wavelength laser pulses. The CARBIDE (Light Conversion) laser was used for the tissue surgery demonstration. The coupling optics used in the experimental setup was explained in section 6.2.

6.3.1. Experimental system for fibre delivered laser pulse ablation of porcine tissue

Figure 6.18. Schematic of the experimental system for fibre delivered pulses to tissue resection
Figure 6.18 shows the experimental system for the tissue resection using fibre delivered ultrashort pulses (USPs). An industrial laser (CARBIDE) with a central wavelength of 1028±5 nm, a range of base repetition rates from 60 kHz to 1 MHz and a tunable pulse width ranging from 232 fs to 10 ps was available. A maximum average power of 5 W and a base repetition rate of 60 kHz was used for the application. The experiment was conducted at 6 ps pulse width regime to mitigate the nonlinear effects in the fibre when delivering high peak power pulses in the femtosecond regime (as reported in section 6.2). The axial displacement of the tissue was achieved by a manual lab jack connected with a dial gauge to find accurate focus shown in figure 6.19. The fibre end probe (consisting of focusing optics) is attached to the movable arm of the multi-axis robotic device (AxiDraw V3) as shown in figure 6.19.

Figure 6.19. The manual jab jack and dial gauge system to adjust the ‘z’ movement of sample

6.3.2. Experimental methodology

6.3.2.1. Scanning strategy with the multi axis robot

This experiment was mainly focused on assessing the ablation depth and thermal damage according to the change in laser pulse energy and average laser fluence. The average laser fluence is calculated from the following formula [6.11-13]

\[
F = \frac{E_p}{\pi \omega_0^2}
\]

(6.5)

where \(E_p\) is the picosecond laser pulse energy and \(\omega_0\) is the radius of the laser spot at the focus.
In the HC-NCF delivered laser pulse resection experiments on the colon tissue samples, the fibre end probe was connected to the XYZ multi-axis robotic device controlled by a software package (Inkscape). Spiral scan patterns of 1 and 2 mm diameter were applied to the surface of the porcine colon. This experiment was carried out at 10 kHz PRR (with a translation speed along the spiral of 13 mm/s) to achieve approximately 90% pulse overlap in laser line separation and scanning direction (to match that used in the galvanometer experiments explained in chapter 3 and 4). Three outward spiral patterns were performed on the tissue surface with laser fluences of approximately 21, 14 and 7 J/cm\(^2\) (28, 18.5 and 9.5 µJ pulse energies) respectively. These parameters therefore replicated similar pulse overlap and laser fluences demonstrated in the galvanometer trials allowing direct comparison of the results. For the 2 mm pattern laser fluences of 21, 17.5 and 14 J/cm\(^2\) were used. These parameters were selected to show the surgical capability of fibre delivered pulses.

6.3.2.2. Development of fibre end probe

The fibre end probe consists of a 76.2 mm long slotted lens tube, a fibre connector and its adaptor, retaining rings and a half inch plano convex lens with 20 mm focal length, of which a 3D model using solid works software is shown in figure 6.20. The cleaved output end of the 1.5 meter long HC-NCF was connected to the fibre connector and was attached to the lens tube using an adaptor. The components of the system are shown in figure 6.20. The lens was placed approximately 65 mm away from the tip of fibre, the light from the fibre was focused down to 13 µm (1/e\(^2\)). This setup was attached to the multi-axis robotic device to achieve the laser beam scanning. Intensity profiles for 2 perpendicular axes through the focused laser spot are shown in figure 6.21 a and b respectively. A spiricon CCD beam profiler was used to find the waist diameter of the focused laser spot.

![Figure 6.20. Schematic of the fibre end probe](image)
Figure 6.21 The horizontal and vertical line scans of focused spot of the laser with a beam waist ($1/e^2$) diameter of 13 µm.

### 6.3.3. Demonstration of porcine colon tissue resection

In order to demonstrate a more surgically relevant modality, the demonstration of tissue ablation was carried using the flexible HC-NCF to deliver pulses to tissue via a moving multi-axis robotic device. In the tissue resection experiments based on HC-NCF, the maximum laser pulse energy measured after the focus lens in the fibre end probe was approximately 28 µJ. Using the spiral scanning pattern described above tissue resection was performed with the fibre delivered pulses. A repetition rate of 10 kHz was used. It is evident from the profilometric and histology analysis (figure 6.22) of the laser ablated tissue, the laser pulses through the HC-NCF are able to excise the biological tissue precisely. The collection and preparation of fresh colon tissue was explained in chapter 3.
Figure 6.22. The 3D profilometric image of laser ablated area using HC-NCF delivered pulses on porcine colon samples and histology results of laser ablated area in different tissue sample with same laser parameters a, d) 21 J/cm$^2$ b, e) 14 J/cm$^2$ and c, f) 7 J/cm$^2$ laser fluences. The black line on the histology image is an artefact after the preparation of sample for histology. The expanded view of maximum thermal damage in each laser ablated tissue area is presented at the bottom of the figure. The repetition rate used was 10 kHz.
The images in figure 6.22 d, e and f are the H&E stained images of laser resected sections, which demonstrate that the maximum width of thermal necrosis was always less than 85 \( \mu m \) in this experiment. In colon tissue, thermal damage of approximately 84 \( \mu m \), 65 \( \mu m \) and 30 \( \mu m \) are observed in laser fluence operations of 21 J/cm\(^2\), 14 J/cm\(^2\) and 7 J/cm\(^2\) respectively. The white regions on the 3D images are missing data points. The maximum width of thermal damage is marked in the corresponding zoomed images (the bottom three pictures of figure 6.22). Nevertheless, the results presented, and in particular the ablation represented in figure 6.22c and figure 6.22f, clearly demonstrate that it is possible to perform selective tissue surgery with negligible tissue damage (down to 30 \( \mu m \)) via the HC-NCF delivered pulses. The highest average ablation depth of 169±56 \( \mu m \) was achieved at 21 J/cm\(^2\) laser fluence and the lowest ablation depth of 87±28 \( \mu m \) (profilometric measurements), was achieved at 7 J/cm\(^2\) laser fluence. The samples used for the 3D profilometry were geometrically distorted after the glutaraldehyde fixation,
which is evident in figure 6.22 a and b. In the fibre delivered pulse ablation experiment, the removal of mucosal tissue was achieved. This depth of ablation is commensurate with the region in the colon where the early stage cancerous polyps develop [6.14-15]. Also, these fibre delivered results compare well with those produced using the galvanometer scheme (refer section 3.5.1 of chapter 3). However, it should be noted that there is a difference in ablation depth, when using approximately similar fluences, between the direct (galvanometer) and fibre delivered laser experiments which is attributed to the difference in depth of focus and applied pulse energy. The galvanometer uses a 100 mm focal length lens whereas a 20 mm focal length lens is used for the fibre delivered laser ablation. In addition, it should be highlighted that the diameter of the raster scan pattern performed by galvanometer was twice that of the diameter of the raster scan pattern performed by the multiaxis fibre scanner. Nevertheless, the results presented here are a clear demonstration that this precision surgical modality could be transferable to the clinic using a delivery scheme based on HC-NCF. Figure 6.23 illustrates the actual system for tissue resection.

Figure 6.24. The Histology images of laser ablated area (a, b & c) using HC-NCF delivered pulses of porcine colon samples and a microscopic image showing the 2 mm diameter ablation pattern on the tissue surface (d) with a) 21 J/cm² b) 17.5 J/cm² and c) 14 J/cm² laser fluences.
The images in figure 6.24 a, b and c show the histology of a 2mm diameter spiral scan pattern with approximately 80% pulse overlap in the laser line spacing and approximately 90% pulse overlap in the laser line scanning direction. Figure 6.24d shows the microscopic 2D image of a 2 mm ablation pattern performed on the surface of a porcine tissue. The laser pulse energy was set at 28, 23.5 and 18.5 µJ and the corresponding fluence was 21, 17.5 and 14 J/cm². From the histology image it is evident that the depth of ablation was changing according to the decrease in applied laser fluence. The maximum width of the necrotic tissue in this experiment was less than 60 µm. These experiments mimic the potential movements of the fibre during a procedure and demonstrates a practical way of delivering the laser pulses in theatre to the surgical site from the laser system (which would be located remotely from the operating table).

6.3.4. Demonstration of cancerous and healthy mouse colon tissue ablation

To demonstrate further the potential of this technique, cancerous and healthy mouse colon were resected using HC-NCF delivered 6ps laser pulses. Laser fluences of 21, 14 and 7 J/cm² were used to ablate 1 mm² spiral pattern on mouse colon tissue. Figure 6.25 shows the morphological changes in the cancerous and healthy colon tissue. The mouse colon tissue is small in size and thin (<500 µm) and to handle the tissue for ablation was a challenge. The mucosa, submucosa and muscularis propria in the healthy colon tissue changed to a more uniform tissue pattern in the cancerous tissue as shown in figure 6.25 a. The laser resected cancerous tissue is presented in figure 6.26. At a fluence of 21 J/cm² all the tissue layers were resected and hole was created in the tissue with minimum thermal damage. Figure 6.27 represents the healthy colon tissue ablation using 21 and 14 J/cm² laser fluences. Unfortunately, the third pattern at 7 J/cm² was missed during the sectioning of tissue for histology due to the small size of the sample which provided a significant challenge for the pathologist. These ablation experiments clearly demonstrate the HC-NCF delivered picosecond laser’s capability to create minimal thermal damage width (<50 µm) in both healthy and cancerous mouse tissue.
Figure 6.25. a) Cancerous colon tissue b) healthy colon tissue

Figure 6.26. Histology results of picosecond laser ablated area in cancerous mouse colon sample with applied laser fluences of a) 21 J/cm$^2$ b) 14 J/cm$^2$ and c) 7 J/cm$^2$
6.4. Conclusions

It has been demonstrated that the seven cell HC-NCF can transmit 2 to 6 ps laser pulses with a maximum input pulse energy of 42 µJ pulse energy (7 MW peak power). The delivered pulses are single mode and with a maximum output pulse energy of 29 µJ. An efficiency (output energy/input energy) of 70% is possible and the spectral and temporal quality of the pulse is preserved. For input pulse energies above 42 µJ the coupling efficiency was shown to decrease due to the onset of damage with total destruction occurring at an input pulse energy of ~70 µJ. This could be mitigated by a better match between the numerical aperture of the lens and fibre and the focused laser spot size and the MFD of the fibre. The fundamental mode of the laser was shown to be well confined in the core with approximately 15 µm mode field diameter. However, this fibre becomes non-linear in nature when transmitting laser pulses with a pulse width less than 2 ps. It was demonstrated that for pulses shorter than 1 ps, non-linear effects can occur causing spectral and temporal changes to the delivered pulses. Supercontinuum generation in the HC-NCF was demonstrated by transmitting pulses with 600 fs pulse width and an input pulse energy of 41 µJ. Therefore this seven cell HC-NCF is not suited for femtosecond laser delivery. It was therefore established that for practical application (i.e. for demonstration of surgery) pulse widths greater than 2 ps and pulse energies less than 42 µJ should be used to maintain a constant efficiency of 70% without any effects of
nonlinearity. Nevertheless, despite these restrictions on the input laser pulse these parameters are sufficient to create precise surgical effect on the tissue surface as demonstrated in section 6.3.3.

In these experiments the potential for using HC-NCF delivered ultrafast laser pulses in a more practical configuration, representative of that which might be employed in an operating theatre for tissue surgery was demonstrated. A 1 mm diameter spiral pattern with a maximum average depth of 169±56 μm was achieved using 21 J/cm² laser fluence and 10 kHz PRR. Minimum average depth of 87±28 μm achieved at 7 J/cm² laser fluence and 10 kHz PRR. The result of 10 kHz laser ablation with 2 mm diameter pattern is also presented, showing minimal thermal damage (< 80 μm) in the resected region. The depth of ablation commensurate with the applied laser fluence in fibre delivered pulse ablation. This indicates that this fibre based laser scalpel has the capability to excise early stage lesions in the inner lining of the bowel. The work demonstrates the capability of HC-NCF delivered picosecond laser in cancerous and healthy colon tissue resection with minimal thermal damage (mouse model). The rate of ablation in this study is low because of the relatively low repetition rate operation of the laser compared to 20 and 40 kHz PRR. However, higher ablation rates can potentially be achieved by higher PRR operation of the laser with appropriate spatial and temporal separation of the laser pulses to avoid the pulse to pulse heat accumulation, which is discussed in chapter 2 and 3. In conclusion, the work in this chapter has successfully demonstrated a way towards implementing the ps laser surgery in a minimally invasive modality via HC-NCF.

6.5. References


Chapter 7

Conclusions and future work

7.1. Conclusions

The ability of ultrashort pulsed picosecond lasers to perform high precision resection of colon and lung tissue has been successfully demonstrated in ex-vivo animal models. The work presented in this thesis has shown the potential viability of picosecond lasers in precision surgery, where minimal thermal necrosis is paramount, by conducting a series of pre-clinical, laboratory studies. Different laser parameters were tested for the tissue ablation to optimize the parameters for efficient colon resection.

Using a 1 kHz PRR, the fluence values of 12 to 4 J/cm² were tested with an observed average depth of resection being 309±43 µm and 122±7 µm for the maximum and minimum fluences respectively. It was also observed that the ablation depth is proportional to the applied pulse energy/fluence with <50 µm thermal damage which is important as it provides a higher level of control for the process. The ablation depth can be dialled in using the pulse energy which is in contrast to existing electrocautery tools where the tissue resection depth is dependent on the level and dwell time of the current. This current is controlled real-time by the surgeon (using a foot pedal) which leaves the possibility of resecting too deep into delicate structures causing complications (such as bowel perforation). By predetermining the laser ablation depth (by pre-setting the pulse energy) the likelihood of removing too much tissue is essentially eliminated.

However, it was observed that for 10 kHz PRR operation (for 1030 nm), shock wave induced mechanical/thermal damage occurred in the ablation zone with less than 12 J/cm² laser fluence (107 µJ pulse energy). At higher pulse overlaps, of 20 kHz and 40 kHz PRR (94 to 88% pulse overlap), the undesirable effect of cavitation and thermal damage on the surgical zone is observed at 88.5 µJ pulse energy operation. These detrimental effects are harder to predict and hence can reduce the accuracy and control of the ablation process.

To prevent the cavitation and mechanical damage, another scanning strategy has been developed with 0% and 20% pulse overlap in the laser scanning direction of an Archimedean spiral pattern. This scanning methodology shows great promise as it exhibits a significant reduction of thermal damage at the resection area whilst obtaining a precise size, shape and depth of the ablated region.
A further set of experiments were carried out using 515 nm wavelength laser with reduced focused spot size (20 µm) compared to the experiments presented with 1030 nm laser (34.4 µm). Using 1 kHz PRR, laser fluences of 19 to 4 J/cm² were tested and it was found again that the ablation depth is proportional to the applied fluence. The maximum thermal damage observed was around 150 µm at 19 and 15 J/cm² laser fluence. This shows that the thermal damage has a relation with applied laser fluence/ pulse energy. In 10 kHz PRR, 515 nm laser ablation, zero thermal damage was observed because of relatively low pulse energy of operation compared to 1030 nm (see figure 4.5 b5). Another set of laser ablation trials have been performed with a very high (97%) pulse overlap in the laser scanning direction and 90% overlap in the laser line separation direction. The results produced with 10, 20 and 40 kHz are not promising for a precision colon resection. In contrast the 0% and 20% pulse overlap spiral patterns generate an ablated crater with very low thermal damage (<60 µm) and a well-defined shape and depth (up to the muscle layer).

The 515 nm picosecond laser was also used to investigate the resection of cancerous and healthy lung tissue in an ex-vivo ovine (sheep) model. It was found that the ablation depth of cancerous tissue is approximately half of the ablation depth of the healthy tissue with the application of same laser parameters in both tissues. This depth of ablation difference is due to the morphological differences between cancerous and healthy tissue. The stiffness of the cancerous tissue is higher when compared to the stiffness of the healthy tissue (by a factor approximately 2), this is discussed in section 5.2 of chapter 5. A 5 kHz and 10 kHz PRR were used for the ablation studies and the results and again showed that ultrashort pulsed picosecond laser ablation provides an effective tool to resect cancerous tissue.

In order to demonstrate that such pulses optimised for tissue resection could be flexibly delivered via an optical fibre, investigations using a hollow core micro-structured fibre (produced by the University of Bath) were conducted. It was shown that the HC-NCF used can transmit 6 ps laser pulses delivering a pulse energy of up to 29 µJ single mode with a 70% coupling efficiency and no change to the spectral and temporal quality of the pulse. The suitability of fibre delivered ultrafast laser beams for tissue surgery was then established using an experimental set-up to mimic a potential practical situation in an operating theatre. The laser was located a few metres away from multi-axis device which locally manipulated the fibre distal end over the surface of a tissue sample. Using this set-
up a maximum average ablation depth of 169±56 μm was achieved at 21 J/cm² laser fluence and a minimum average depth of 87±28 μm achieved at 7 J/cm² laser fluence. In this experiment it was also demonstrated that the maximum width of thermal necrosis is < 85 μm. The relationship between laser fluence and ablation depth are very similar for both direct laser ablation and fibre delivered laser ablation hence it is concluded that by implementing a flexible fibre delivery modality no loss of performance is observed. The achieved main results of 1030 nm and 515 nm colon tissue resection using picosecond laser is presented in tables 7.1, 7.2 and 7.3.

Wavelength 1030 nm; spot size ~13 μm- HC-NCF based tissue resection (Chapter 6)

LLD-Laser line separation direction
LSD- Laser scanning direction

<table>
<thead>
<tr>
<th>PRR</th>
<th>Pulse overlap (%)</th>
<th>Pulse energy (µJ)</th>
<th>Laser fluence (J/cm²)</th>
<th>Thermal damage (µm)</th>
<th>Secondary effects of plasma formation</th>
</tr>
</thead>
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<tr>
<td>LLD</td>
<td>LSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kHz</td>
<td>90</td>
<td>90</td>
<td>28</td>
<td>21</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>18.5</td>
<td>14</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>7</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
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Table 7.1. Fibre delivered ultrashort pulse picosecond 1030 nm Carbide laser resection results using different pulse energy, laser fluence.
<table>
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<tr>
<th>PRR</th>
<th>Pulse overlap (%)</th>
<th>Pulse energy (µJ)</th>
<th>Laser fluence (J/cm²)</th>
<th>Thermal damage (µm)</th>
<th>Secondary effects of plasma formation</th>
</tr>
</thead>
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<tr>
<td>1 kHz</td>
<td>90</td>
<td>107, 88.570, 5.525 and 34.5</td>
<td>12, 10, 8, 6 and 4</td>
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<td>No</td>
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<tr>
<td>10 kHz</td>
<td>90</td>
<td>107, 88.5 and 70.5</td>
<td>12, 10, and 8</td>
<td>High mechanical/thermal damage</td>
<td>Shock Wave induced mechanical/thermal rupture</td>
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<tr>
<td>10 kHz (Spiral) Carbide</td>
<td>90</td>
<td>46 and 33</td>
<td>18 and 13</td>
<td>Moderate thermal damage &lt;100</td>
<td>No severe effects but moderate thermal effects were present</td>
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<tr>
<td>20 kHz</td>
<td>90</td>
<td>107, 88.5, 70.5, 52.5 and 34</td>
<td>12, 10, 8, 6 and 4</td>
<td>Severe damage</td>
<td>Cavitation/gas bubble, shock wave and heat accumulation</td>
</tr>
<tr>
<td>20 kHz</td>
<td>94</td>
<td>88.5</td>
<td>10</td>
<td>Severe damage</td>
<td>Cavitation/gas bubble, shock wave and heat accumulation</td>
</tr>
<tr>
<td>40 kHz</td>
<td>94</td>
<td>88.5</td>
<td>10</td>
<td>False</td>
<td>Tissue bulge (no histology)</td>
</tr>
<tr>
<td>20 kHz (spiral) Carbide</td>
<td>90</td>
<td>33</td>
<td>13</td>
<td>&lt;60</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 7.2. Ultrashort pulse picosecond 1030 nm laser resection results using different pulse overlap, pulse energy, laser fluence and PRR. Wavelength 1030 nm, Spot size (Trumpf): 34.4 µm, Spot size (Carbide): 18 µm. (Chapter 3)
<table>
<thead>
<tr>
<th>PRR</th>
<th>Pulse overlap (%)</th>
<th>Pulse energy (µJ)</th>
<th>Laser fluence (J/cm²)</th>
<th>Thermal damage (µm)</th>
<th>Secondary effects of plasma formation</th>
</tr>
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<tbody>
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<td>1 kHz</td>
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<td>90</td>
<td>58.57, 47.6, 36, 24.17 and 13.27</td>
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<td>90</td>
<td>58.57, 47.6, 36, 24.17 and 13.27</td>
<td>19, 15, 11, 8 and 4</td>
<td>&lt;150</td>
</tr>
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<td>19, 15, 11, 8 and 4</td>
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<tr>
<td>10 kHz</td>
<td>97</td>
<td>90</td>
<td>61.32, 57.9, 54.82, 51, 47.6 &amp; 43.85</td>
<td>19.5, 18, 17, 16, 15, 14</td>
<td>&lt;150</td>
</tr>
<tr>
<td>40 kHz</td>
<td>97</td>
<td>90</td>
<td>61.32, 57.9, 54.82, 51, 47.6 &amp; 43.85</td>
<td>19.5, 18, 17, 16, 15, 14</td>
<td></td>
</tr>
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</tr>
<tr>
<td>40 kHz</td>
<td>90</td>
<td>0</td>
<td>40</td>
<td>13</td>
<td>&lt;60</td>
</tr>
</tbody>
</table>

Table 7.3. Ultrashort pulse picosecond 515 nm laser resection results using different pulse overlap, pulse energy, laser fluence and PRR. Wavelength 515 nm, Spot size (Trumpf): 20 µm. (Chapter 4)
Overall the work in this study has demonstrated that an ultrashort pulse laser system in combination with a HC-NCF has significant potential for the development of a new surgical tool. Such a fibre-based laser scalpel would have the capability to precisely excise early stage lesions in the inner lining of the bowel (and possibly other indications - see Future Work). In conclusion, it is clear that a promising route towards implementing ps laser surgery in a minimally invasive modality via HC-NCF has been demonstrated.

7.2. Future Work

The ultimate aim of this translational research is to actualise the colonoscopic/endoscopic surgery in a human based on HC-NCF delivered ultrashort picosecond laser pulses. The viability of using the picosecond laser in colon tissue resection and the efficient delivery of laser pulses through HC-NCF has been demonstrated. However, now that the potential of the fibre delivered pulses for precision tissue resection has been shown there are a number of technological challenges that remain in order to implement a real device in clinical procedures which are clearly beyond the scope of this study.

7.2.1. Miniaturised distal end optics

In the experiment with the multi-axis stages, a basic focusing optic arrangement was used with a fibre coupler that provided a stand off to a convex focussing lens. Clearly, in order to realise a device deployable via an endoscope, a miniaturised version of a lens-based laser focusing end probe with large depth of focus and with the additional capability to locally manipulate or scan the fibre end, must be developed. There are number of works that report technologies aimed at realising such devices [7.1-2]

In addition to standard focusing lenses the development of an ultrashort pulsed picosecond laser focusing system using axicon lenses could potentially be beneficial. The Gaussian beam focusing via an axicon lens produces a large depth of focus with an extended region of intensity and enough laser fluence to ablate the tissue. The most efficient technique to create a Bessel beam is to illuminate the axicon with a Gaussian beam. The HC-NCF used here is capable to produce Gaussian beam at higher laser pulse energy and fluence and hence might be compatible with an axicon based distal end probe. Works have been reported which show the application of Bessel beams in hard tissue ablation [7.3] and material processing [7.4].
In 2008, Hoy et al. developed a hollow core fibre scanning system based on a two axis micro-electromechanical system. The fibre end probe was used to create lissajous patterns on a tissue phantom [7.2]. A piezoelectric tube actuator was attached to the optical fibre and was used to create lissajous, spiral and circular patterns on samples according to the voltage and phase difference applied to the piezoelectric actuator [7.5-6].

### 7.2.2 Imaging and targeting systems

Clearly such a precise tool needs the capability to precisely target the cancerous or diseased tissue. Within the research group at Heriot-Watt University and in collaboration with clinical partner at the University of Leeds, Dr. Rainer Beck is working towards the development of selective ablation of cancerous tissue in mouse model based on fluorescence markers. The effective and selective removal of cancerous tissue is possible with marking of the lesion with a fluorescent marker. Fluorescence marking and imaging technique is widely used for the early stage detection of colon cancer [7.7-9]. The idea is to compare the fluorescence from the surgical area before and after the surgery to ensure the complete removal of cancer tissue, keeping the healthy tissue intact. Figure 7.1 shows the difference between images of cancerous tissue imaged using a white light endoscopy and a fluorescence endoscopy in the colon when a fluorescent agent has been applied [7.10].

![Colon cancer imaging](image)

Figure 7.1. Colon cancer imaging. White light endoscopy (left) and fluorescence endoscopy (right). The cancerous part of the tissue is glowing because of the fluorescent marker [7.10].
7.2.3 Translation to other Applications

The techniques developed in this thesis have the potential to be translated to address a wide range of challenges in other clinical areas. For example, the application of the picosecond laser in porcine brain tissue surgery has been demonstrated by Goetz et al. in 1999 [7.11]. A multiple coaxial tube with lenses attached to a stereotactic frame was used to deliver laser to the tissue. Also, femtosecond laser ablation of bovine neural tissue also presented in [7.12] and more recently, Kerse et al. demonstrated rat brain tissue ablation using burst pulses of a femtosecond laser [7.13]. However, the lack of delivery mechanism limits the minimally invasive application of ultrashort pulsed lasers in brain surgery. Therefore it is proposed that the combination of HC-NCF with an ultrashort laser (as demonstrated in this thesis) and implementation of a fluorescence imaging scheme could be an outstanding choice for brain surgery provide very high precision and control with the benefit of being able to enable greater access using fibre delivery.

The use of picosecond/femtosecond laser in vocal fold surgery is described in section 2.3 of chapter 2 and femtosecond laser based eye surgery is already an established method [7.14]. Both these surgical modalities could be improved by using flexible delivery of laser pulses as described in this work.

Finally, the feasibility of using picosecond laser based surgery of healthy and cancerous porcine lung tissue is presented in chapter 4. This is currently an unexplored area of ultrashort pulsed laser surgery and once again the ability to flexibly deliver such pulses within the confines of the human lung could have a significant impact on the successful treatment of diseases such as cancer.

7.3. References


