University of Southern Queensland
Faculty of Engineering and Surveying

Welding of Skin using Nd:YAG Laser with Bipolar Contact Applicators

A dissertation submitted by
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2003

In fulfilment of the requirements of

Master of Engineering

Certification of Dissertation

I certify that the ideas, designs and experimental work, results, analysis and conclusions set out in this dissertation are entirely my own efforts except where otherwise indicated and acknowledged.

I further certify that the work is original and has not been previously submitted for assessment in any other course or institution.

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Lynette M Brodie (Candidate) Date

_____ _____

Professor John Grant-Thomson (Supervisor) Date

Abstract

This thesis investigates the feasibility of closing wounds in skin tissue by laser welding as a substitute for suturing. Such a process would provide advantages in some surgical procedures. The investigation revised available theory on the action of lasers on skin tissue as a basis for the experimental program. The results of experiments using rat skin are then reported. In addition a thorough investigation of the normal (uninjured) tensile strength of rat skin was undertaken to provide a base line comparison..

Laser systems permit very high-energy radiation of a single wavelength to be focused on a tiny spot, and have found application in many areas of engineering. They are also currently used in many branches of medicine including ophthalmology, gynaecology, dermatology, otolaryngology, and gastroenterology. These medical applications employ argon, YAG, and carbon dioxide type lasers. In many cases, lasers have been found to be more effective than conventional treatment methods with advantages including reduced blood loss, more accurate removal of unwanted tissue, shorter operating times and less postoperative pain and care (Gibson and Kernohan, 1993).

Tissue welding using laser energy represents a small but growing area of medical research and is largely focused on anastomosis. This thesis investigates, using a specific experimental program, the feasibility of the bipolar contact Nd:YAG laser to weld **cutaneous** tissue. No similar published research has been identified in this area. The available literature focuses on non-contact lasers of various types and settings and mainly in the area of anastomosis. The experimental methodology and the specific technique for the bipolar contact laser is developed, tested and evaluated as part of this project.

The welding techniques developed in this project overcome the previous difficulties of tissue alignment. The use of the bipolar laser probes substantially improves the ability to align the tissue

edges to be joined. The probes give tactile feedback to the user and the pressure effect of the probes may assist with the welding process. The developed technique was no faster or easier than suturing.

Viable welds and a useable technique for welding skin on rats were developed and tested. The resultant healing was comparable with published literature and both sutured and welded wounds returned to full strength as compared with the baseline data collected. All wounds had returned to full strength within 91 days. At 75 days there was not significant difference between laser welded and sutured wounds and they had achieved approximately 90% of full strength. Time to half strength was approximately 42 days and there was a larger standard deviation for both laser welded and sutured wounds. The most significant increase in strength and therefore healing occurred in the first 42 days. Simhon et al, 2001 states that a tensile strength of 0.6 N ± 0.4 N was sufficient to hold tissue together. By day 7 the strength of the wound (laser welded) was more than twice the strength needed to maintain closure. There may have been sufficient healing for this to occur earlier but there were insufficient animals to allow for testing of this theory.

In conclusion this experimental program and investigation has reviewed the available literature on the current use of lasers in medicine and their specific laser-tissue interaction which leads to tissue welding. It has provided a large database of tensile strength measurements collected with a reproducible methodology and reported in a standardised format. The developed technique for laser tissue welding using a bipolar contact Nd:YAG laser has been established and verified. It produces viable welds comparable in strength and healing rates to sutures.

Acknowledgements

There are numerous people who have contributed either directly or indirectly to this work. I would like to thank firstly my family, my husband Ian and daughters Ellen and Megan for their continuous support over many years.

I would also like to acknowledge my supervisor Dr John Grant-Thomson; Dr Ross Harrington and Dr Geoff Buckham for the use of the laser and operating rooms at St Andrews Hospital; Dr Ron Atkinson for his assistance with the animals and their care; the staff of the USQ Animal House; Dr Brian Harmon from Queensland University of Technology (QUT) for the preparation of the histology slides and Dr Deb Stenzel also from QUT for the histological analysis.

There were also numerous colleagues in the Faculty who gave encouragement to complete the project: Dr John Leis, Dr Mick Morgan and Mr Doug Baddeley. Lastly the acknowledgements would not be complete without a special thank you to Dr Mark Porter for his enduring confidence, support and assistance.

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1 General Introduction

This thesis investigates the feasibility of closing wounds in skin tissue by laser welding as a substitute for suturing. Such a process would provide advantages in some surgical procedures. The investigation revised available theory on the action of lasers on skin tissue as a basis for the experimental program. The results of experiments using rat skin are then reported.

1.1 Background

Laser systems permit very high-energy radiation of a single wavelength to be focused on a tiny spot, and have found application in many areas of engineering. They are also currently used in many branches of medicine including ophthalmology, gynaecology, dermatology, otolaryngology, and gastroenterology. These medical applications employ argon, YAG, and carbon dioxide type lasers. In many cases, lasers have been found to be more effective than conventional treatment methods with advantages including reduced blood loss, more accurate removal of unwanted tissue, shorter operating times and less postoperative pain and care (Gibson and Kernohan, 1993).

Today the cost of medical care and surgery is an increasing concern to governments and society. Research efforts are focusing on equipment, treatment and techniques that result in faster healing times and shorter hospital stays. Patients also prefer techniques that result in less pain and scarring.

`Tissue welding` using laser energy represents a small but growing area of medical research and is largely focused on `anastomosis`[1]. This thesis investigates, using a specific experimental program, the feasibility of the bipolar contact Nd:YAG laser to weld **cutaneous**

[1] Words in **`courier new`** font are defined in Glossary.

tissue. No similar published research has been identified in this area. The available literature focuses on non-contact lasers of various types and settings and mainly in the area of anastomosis. The experimental methodology and the specific technique for the bipolar contact laser is developed, tested and evaluated as part of this project

1.2 Project Objectives

The main project objective is to experimentally investigate the feasibility of using a laser for welding cutaneous tissue. The feasibility of the process will be quantified by comparisons of welded cutaneous rat skin and sutured skin with uninjured rat skin.

In order to achieve this objective, several major aims were identified:

- To investigate the current uses of lasers in medicine.
- To assess the types of lasers suited to laser-tissue interaction and to determine a suitable type for use in this project.
- To evaluate known theory and published experimental work on the effects of laser energy on cutaneous tissue.
- To design and undertake an experimental program to test the effectiveness of laser welding skin in promoting healing.
- To compare the healing process of skin following laser welding with that of suturing.

The first three of these aims require an overview of the major medical fields as well as the enormous range of currently available commercial lasers. Figure 1.1 indicates the current range of lasers in commercial use and the proportion of these in medical use. They form the fourth largest section of sales in the industry.

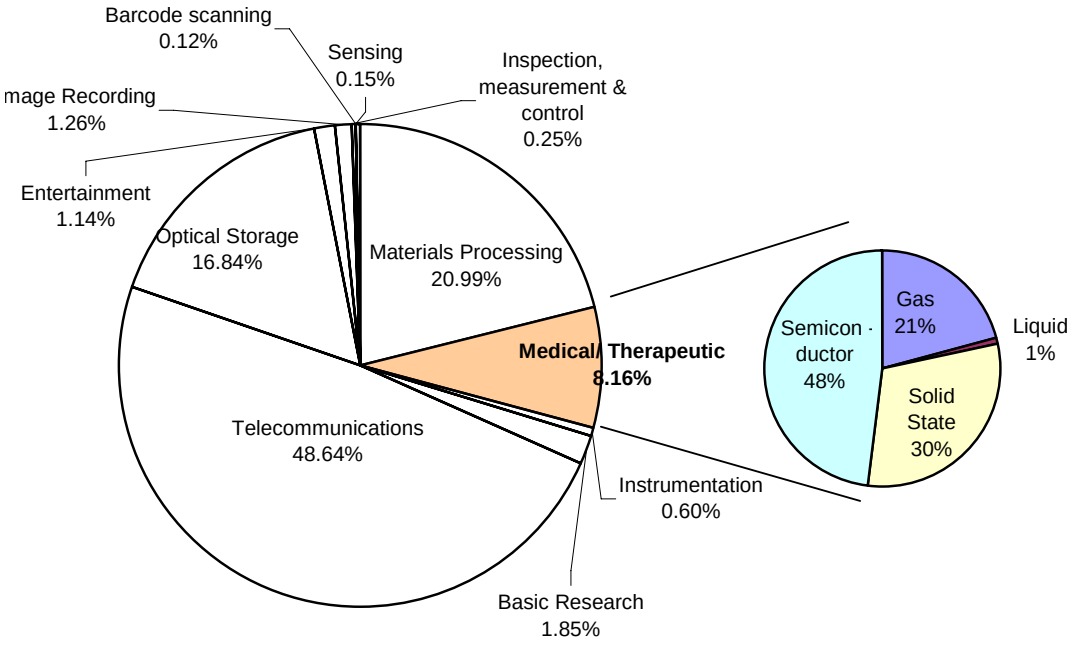


Figure 1-1 Laser sales 2000-2001

The approach used in this thesis is to define the major areas of medicine covering all areas where the use of lasers is well established (e.g. Ophthalmology and Dermatology) through to areas where the use of lasers is just emerging. The details of medical procedures are outside the scope of this thesis and so the discussion is limited to the type of laser used and the resulting effect on tissue.

Tissue welding is included in the major review of lasers in medicine. It meets the second and third aims of the thesis and concludes that a Nd:YAG bipolar contact laser has potential for welding cutaneous tissue. An experimental program is described for the testing of weld strength and healing time for 20 female inbred Wistar rats matched in age and general health. The rats received two scalpel skin wounds, one of which was conventionally sutured while the second was laser welded using a technique developed specifically for this project. Tensile tests were used to

determine the baseline tensile strength of uninjured skin to compare sutured and laser welded skin healing times.

1.3 Thesis Organisation

This thesis spans two disciplines of engineering and medical science.

Background information relating to lasers, including brief history of development, laser classification, safety issues and laser-tissue interaction are covered in Chapter 2.

Chapter 3 contains a literature review on Tissue Welding. It indicates that although significant work has been done on anastomosis, there has been little experimental work done on welding of cutaneous tissue. In particular a bipolar contact laser had never been used to undertake this kind of work.

Chapter 4 investigates the history of Nd:YAG bipolar contact laser as well as the effect of this particular wavelength on tissue with contact and non contact delivery methods.

Experimental methodology, results and analysis, discussion and conclusion pertaining to the second objective are contained in Chapters 5, 6, and 7 respectively.

2 Lasers – Overview and use in medicine

This review provides a brief introduction to the main terms general to all lasers. It covers the history and the classification of laser systems. Lasers can inflict serious and permanent injury to operators, clinical nursing staff and patients and a section is devoted to appropriate laser safety procedures. The use of lasers in medicine is summarised and the theory of laser – tissue interactions is discussed.

2.1 Basic laser operation

Laser light exhibits unique properties. The stimulated emission can be powerful and covers radiation in the `electromagnetic spectrum` from the infrared through to ultraviolet.

All lasers, regardless of the frequency of the output work, on the same theory of operation and have the same basic components. They can operate in different modes that affect the power delivered to the target medium. However the single most important factor in the effective application of laser is the power concentrated onto a specified area. This is known as the power density or irradiance. The spot size depends on several variables including the focal length of the lens system, the wavelength and the transverse electromagnetic mode. For more details refer to Appendix A - Basic Components and Operation of a Laser .

The diversity of output frequency, power and the operating modes mean that lasers have found wide and varied applications include materials processing, alignment, remote sensing/laser radar, military applications and communications outlined in Table 2-1.

Table 2-1 Current uses of lasers

Research	Study of mechanisms at interfaces Detection of single molecules/particle characterisation Investigation of atomic fusion DNA and blood analysis
Commercial	Bar code and colour scanners Laser pointers Surveying and levelling Remote sensing High speed and high performance reprographics and printing Food processing
Industrial	Materials processing including: Cutting Welding Etching Material curing Photo plotters for PCB (Printed Circuit Board) manufacture Semiconductor inspection equipment
Entertainment	CD players CDROMs (computers) Laser light shows and Holographics
Medical	Virtually all fields of medicine but in particular: Surgery; Ophthalmology; Dermatology; Physiotherapy; Dentistry

2.2 Brief history of the Laser

The word laser is an acronym for Light Amplification by Stimulated Emission of Radiation and today is one of the few acronyms to be accepted as a "word". Lasers are used in a wide range of applications from local supermarkets through to research laboratories. Their development depended on an understanding of the quantum nature of our world and took place over many decades with contributions from many famous scientists.

The principles upon which all laser devices are based were developed at the turn of the century by the physicists Planck and Einstein. During this period a certain amount of speculation and discussion had surrounded Maxwell's concept of considering electromagnetic radiation as waves. It was obvious that the theory had a number of shortcomings and failed to explain some commonly observed phenomena. In order to explain these anomalies, Planck proposed quantal theory: the idea that radiation could be considered as discrete quanta or bundles of energy. Building upon Planck's concept of quantal energies, Einstein published a paper in 1917 entitled "*Zur Quantum Theori der Strahlung"* (On the Quantum Theory of Radiation) which outlined the key principles for the stimulated emission of photons (Baxter, 1994 p6).

However it was another 40 years before a group working at Columbia University produced stimulated radiation. This device was called a MASER:- Microwave Amplification by Stimulated Emission of Radiation (Gordon et al, 1955). In 1958 Schawlow and Townes proposed the development of an "optical maser" based on this previous work[2]. However it was Theodore Maiman in 1959 who first published on stimulated light emission from a ruby crystal, the first laser.

The 1960's saw a rapid development of this initial work by Maiman using a wide range of alternative lasing media, Javan et al (1960) with the elemental gasses of Helium and Neon and Patel (1965) with the CO_2. Further developments in this time included the argon and neodymium-glass and neodymium: yttrium aluminium garnet (Nd:YAG) lasers (Baxter, 1994 p7). From these early beginnings researchers have gone on to develop a multitude of lasing materials each with its own specific wavelength and a variety of output power and mode settings.

[2] In 1977 Gordon Gould won a thirty year legal battle and was granted a patent on laser technology ahead of Schawlow and Townes.

Major developments in the history of lasers are presented in Table 2-2 on page 9, however due to the large range of lasing mediums this table has been limited to significant milestones of development.

Table 2-2 History of development of the laser

Year	Contributor	Contribution
1917	Einstein	Principle of "stimulated emission"
1958	Schawlow & Townes Gould	"LASER" principle
1960	Maiman	Developed Ruby laser
1960	Sorokin and Stevenson	Solid state uranium laser[3]
1960	Javan, Bennett, Herriott	Developed Helium-Neon Laser[4]
1960	Houtermans	Potential of excimer lasers examined
1961	Snitzer	Developed Nd:Glass laser
1962	Hall	Developed semiconductor laser
1964	Sorokin and Lankard	Developed dye laser
1964	Patel	Developed CO_2 laser
1964	Bridges	Developed argon laser
1964	Hellwarth	Described Q switching technique
1964	Geusic, Marcos, Van Uitert	Developed Nd: YAG laser
1966	Sorokin, Lankard	Stimulated emission from organic molecules (Liquid Dye Laser)
1968	Silfvast	Developed metal vapour lasers
1970	Basov, Danilychev, Popov, Khodkevich	Demonstrated (liquid) excimer lasing action
1972	Koehler, Ferderber, Redhead, Ebert	(gas) excimer lasing action
1975	Madey	Developed free electron laser
1984	Matthews	Developed selenium X-ray laser

[3] First new laser following Theodore Maiman's ruby laser
[4] First gas laser

2.3 History of Medical Lasers

The world's first medical laser laboratory was established at the University of Cincinnati in 1961 and was conceived to investigate the *safety* of the then new technology (Baxter, 1994 p7). Shortly after this a laboratory was established at the Children's Hospital of Cincinnati under direction of Professor Leon Goldman to assess the potential surgical application of lasers.

Ophthalmologists were the first medical specialists to successfully use lasers in surgical applications. In 1961, just one year after the invention of the laser, Zaret et al (1961) first published experimental studies on the use of an 'optical maser'. Two years later Campbell et al (1963) were reporting the treatment of patients with retinal detachment. Goldman et al (1964) and Stern and Sognnaes (1964) reported laser use in dentistry. Early laser treatment was limited to the application of ruby lasers but as the development of different lasing mediums continued so to did the investigation of the different laser properties and their potential application into medicine and surgery (Niemz, 1996 p2). Major milestones in the medical laser field are summarised in Table 2-3. It begins with Meyer-Schwickerath in 1954, who first used the high energy of light for surgical procedures, and concludes with the development of the bipolar contact probes which were used in this project. These bipolar probes represented a significant development in medical lasers. This thesis investigates a further use of these probes.

Table 2-3 Development of medical laser technology

Year	Contributor	Contribution
1954	Meyer-Schwickerath	First surgical use of light
1961	Goldman	First ruby laser clinical application
1961	Schnitze	Developed Nd:Glass laser
1964	Patell	Developed CO_2 laser
1964	Geusic, Marcos, Van Uitert	Developed Nd: YAG laser
1965	Polanyi	First CO_2 laser clinical application
1968	L'Esperance	First argon laser clinical application
1977	Kiefhaber	First Nd:YAG clinical application
1980	Dougherty	Described Photodynamic Therapy
1984	Daikuzono, Joffe	Developed Nd:YAG contact technology

2.4 Classification of lasers

In the Australian and New Zealand Standards (AS 211/NZS 5821), laser products are grouped into four classes according to the degree of hazard posed. As the class number increases, more engineering safety features are required from the manufacturer and progressively more stringent administrative safety measures are recommended to the user. The classification of the laser is determined by an `Accessible Emission Limit (AEL).` It is specified for each class of product and refers to the amount of laser radiation emitted by the laser and which a detector with a specified aperture collects. Accessible emission limits are derived from the `Maximum Permissible Exposure (MPE)` levels and used to determine the hazard potential. A table of MPE levels for different lasers is listed in Table B-2 on page151.

An overview of the four major classifications of lasers is summarised in Table 2-4 below and discussed more fully in Appendix B on page 148. Table B-3 and Table B-4 in Appendix B Classification of Lasers and Laser Safety, lists all relevant Australian and New Zealand Standards for laser classification, potential hazard and installation requirements respectively.

Table 2-4 Laser classification overview

Class	Definition
Class I	The least-hazardous class. Considered incapable of providing damaging levels of laser emissions.
Class IIA	Visible Light lasers viewed for a duration of less than or equal to 1000 seconds
Class II	Applies only to visible laser emissions and may be viewed directly for time periods of less than or equal to 0.25 seconds, which is the aversion response time.
Class IIIA	Dangerous under direct or reflected vision. These lasers are restricted to the visible electromagnetic spectrum.
Class IIIB	May extend across the whole electromagnetic spectrum and are hazardous when viewed intrabeam.
Class IV	The highest-energy class of lasers, also extending across the electromagnetic spectrum. This class of laser presents significant fire, skin, and eye hazards.

Most medical lasers are Class IIIB or IV. Class IIIB lasers have an upper power limit of 500mW (intermediate power:- continuous wave (c.w.) 5 – 500 mW or pulsed 10J/cm^2) and have found uses in spectrometry, stereolithography and entertainment light shows. Class IV lasers are high power (c.w. >500mW or pulsed >10J/cm^2) devices. As well as in medicine (surgery) they have found industrial uses in drilling, cutting, welding and micromachining processes.

These two classes of laser pose significantly more safety risks and require greater controls to ensure safe operation.

2.5 Laser Safety

Laser safety for both the patient and operating room staff, including the surgeon, has become a complex issue due to the wide variety of wavelengths, maximum power levels and delivery methods available in today's medical lasers. Hazards from the laser include direct and indirect (reflected) beam exposure; fire hazards; smoke produced by vaporisation (containing pathogen and chemical toxins); as well as the standard safety measures needed for electrical and electronic equipment (Sliney, 1995). These hazards are summarised in Table 2-5.

For laser light to be a hazard to any biological structure it must be absorbed by the tissue where the laser light is converted to heat. Because of this property different wavelengths pose problems to different tissues. Some wavelengths are readily absorbed by skin chromophores or interstitial water, whilst others are transmitted through the structure with relatively little absorption and damage to the tissue.

Table 2-5 Summary of laser hazards

Laser Radiation Hazard	Eye	Corneal or retinal burns (depending on wavelength) Cataracts Retinal injury
	Skin	Skin burns from acute exposure (direct or reflected beam) Skin carcinogenesis (depending on wavelength)
Chemical Hazard	Excimer, dye and chemical lasers contain toxic substances Smoke produced from vaporisation or laser induced reactions	
Electrical Hazard	Danger from high voltage power supplies	
Secondary Hazard	Cryogenic coolant hazards Excessive noise X radiation from high voltage power supplies Fire hazards (beam exposure to flammable substances)	

Medical lasers (Class IIIB and IV) are available in all wavelengths, ultraviolet to infrared, and by definition operate at high power levels. They are a possible hazard to eyes and skin and tissue heating may produce a smoke plume. Potential damage to the skin and parts of the eye are well documented and understood. Eye damage falls mainly into two categories: damage to the retina by visible and near infrared wavelengths and to the cornea by far UV and infrared. The Nd:YAG laser used in the work for this thesis can damage the iris and retina of the eye causing cataract and retinal burns; excessive dry skin and skin burn. These effects and a comparison with other lasers and hazards are detailed in Appendix B, specifically Table B-6 Laser wavelength and eye damage and Table B-7 Summary of possible skin damage by laser radiation.

In a feasibility study for the experimental work done for this thesis a significant smoke plume for heating tissue was noticed. This hazard was investigated for potential risks. It was found that the hazard from the smoke plume has not been well established, with differing results. Relatively little work on the smoke plume from Nd:YAG lasers was identified in the literature. The laser will theoretically only produce a smoke plume if the energy density is high or if insulated chromophores are irridated.

Nezhat et al (1987) examined the compositions of the smoke plume produced during carbon dioxide laser surgery to determine whether the operating room team was at risk from the laser smoke. The authors were interested in calculating the probability that something the size of a whole red blood cell (7.5 μm) would be present in the smoke. Particles with an aerodynamic diameter ranging from 0.1-0.8 μm were found in the collected smoke plume samples but no cell-size particles, including cancer cells, were present in the plume (reported probability 0.000001).

The findings of this study differ from some earlier ones in which intact cells or identifiable cell parts were collected from both carbon dioxide (CO_2) and neodymium: yttrium-aluminium-garnet (Nd:YAG) laser radiation of animal tissue. Nezhat et al (1987) concluded that although no identifiable hazard from airborne cancer cells was detected, a significant portion of the particles in the smoke was in the range of 0.5-5.0 μm. These particles are too small to be effectively filtered by surgical masks. It was recommended that a mechanical smoke evacuator system with a high-efficiency multi-stage filter be used during smoke generating laser vaporisation procedures.

Garden et al (1988) analysed the vapour produced by the vaporisation of papillomavirus infected verrucae. This study concluded that intact viral deoxyribonueleic acid (DNA) was liberated into the air with the plume of laser-treated verrucae. Papillomavirus DNA has been demonstrated to be infectious. Therefore, when performing laser therapy on patients infected with viruses such as hepatitis or the human immunodeficiency virus, the smoke plume should be assumed to be infectious and appropriate precautions. This includes a well maintained vacuum apparatus to evacuate the smoke. Further information of the hazards of smoke plumes produced by lasers is detailed in Appendix C on page 159.

2.6 Lasers and Medicine

Lasers are classified into four main categories by their lasing medium: gas, liquid, solid state and semiconductor. Within each of these classifications lasers are known by their specific lasing medium e.g. CO_2 is a gas laser in which the lasing medium contains a specific concentration of carbon dioxide, nitrogen (or argon), and helium in the ratio of 1:1.5:4. Each lasing medium produces a unique wavelength of emitted laser light. The use of different lasers in specific areas of medicine for clinically accepted procedures is summarised in Table 2-6.

Appendix C discusses the four main categories of lasers that have found applications in medicine. In addition the lasing medium, pumping method, mode of operation and where applicable, the delivery method has been further discussed.

Appendix D is a comprehensive literature review of the use of lasers in major areas of medicine. It covers the use of lasers and the resultant laser-tissue interaction in clinically accepted procedures in general surgery, dermatology, urology, neurology, orthopaedics and dentistry.

The type of laser selected depends on the application and hence a discussion of all lasers for all applications is a broad subject. From the viewpoint of a physicist, all lasers share common properties, about which useful generalisations can be made. However from the physicians' point of view, each laser in the medical field is chosen because its emissions produce a unique tissue effect. To select an appropriate laser system for medical usage, it is necessary to understand its biological effect on tissue. For any laser to have an effect on living tissue, it must first be absorbed. If the energy is reflected from the surface of a tissue, or if it is completely transmitted through a tissue, no biological effect will result. Similarly if the energy is scattered within a tissue, the effect will be relatively non-selective and imprecise. To obtain an exact effect within any given tissue, the laser energy must be absorbed by some natural component of that tissue or by the addition of a specifically introduced chromophore e.g. tattoo pigment. This interaction with living tissue is generally a function of the wavelength of the laser system. Hence the extent to which surgeons can generalise about the use of lasers is quite limited (Absten, 1991). A summary of medical lasers, wavelength and operating characteristics is compiled in Table 2-7. This table was used to assist in the selection of laser type for this project.

Table 2-6 Summary of clinical use of lasers in medicine.

Laser	Medical Application
Gas	
Helium-neon	Physiotherapy, targeting beam, biostimulation
Argon	General Surgery; Ophthalmology; Dermatology; Photodynamic Therapy; Tissue Welding; Otorhinolaryngology; Gastroenterology; Gynaecology
CO^2	General Surgery; Gynaecology; Ophthalmology; Dermatology; Tissue Welding; Otorhinolaryngology; Neurosurgery; Dentistry; Orthopaedics
Metal vapour	Ophthalmology; Photodynamic therapy; Dermatology
Excimer	Ophthalmology; Dentistry; Dermatology
Liquid	
Tunable Dye	Dermatology, Photodynamic therapy, Ophthalmology, Gynaecology
Solid State	
Ruby	Dermatology
Nd:YAG	Urology; Gynaecology; General Surgery; Tissue Welding; Otorhinolaryngology; Neurosurgery; Gastroenterology; Orthopaedics
Er:YAG	Dentistry, Ophthalmology, Dermatology; Gynaecology
Ho:YAG	Dentistry; Orthopaedics (Tissue ablation), Gynaecology,
KTP	Gynaecology, gastroenterology, Otorhinolaryngology
Semiconductor	
Diode	Tissue Welding; Ophthalmology, biostimulation

Table 2-7 Summary of laser wavelengths commonly used in medicine

Laser	**Colour**	**Wavelength (nm)**	**Typical power rating (W)**	**Depth of Penetration**	**Typical pulse duration**
Excimer: ArF KrCl KrF XeCl XeF	Ultraviolet UV-C UV-B UV-C UV-B UV-A	 193 222 248 308 351	0.5-50 W	5 to 40μm	10-20 ns
Argon	Blue Green	488 515	3 - 20 W	Approx 0.3-0.5mm	CW
Krypton	Green Yellow Red	531 568 647	1 W	Approx 0.5 to 2mm	CW
KTP	Green	532	5 - 15 W	Approx 0.5 mm	CW - 0.25 ms
Dye laser	Variable with dyes Red Yellow/green	 632 577	1 - 5 W	 Approx 1-2mm Approx 0.5 - 1mm	CW or pulsed
Gold vapour	Red	628	10 W (average)	Approx 1-2mm	CW
Helium neon	Red	632	1 - 30mW	Approx 1-2mm	CW
Ruby	Deep Red	694		Approx 1-2mm	1-250 μs
Nd:YAG	Near Infrared (IR-A)	1064 1318	1 - 120 W	2-6mm	CW or 30-100 ps
Er:YAG	Near Infrared (IR-B)	2940	10J	2-6mm	Pulsed
Ho:YAG	Near Infrared (IR-B)	2100	30 W	2-6mm	CW & Pulsed
Carbon dioxide	Far Infrared (IR-C)	10600	1 - 80 W	< 0.5mm	CW or pulsed
Diode laser	Variable (Red to Near Infrared, IR-A)	670-900	1 - 4 W	Approx 2-3mm	CW or pulsed
Alexandrite	Near Infrared (IR-A)	720-800	KW	Approx 2-3mm	50ns-100 μs

2.7 Delivery Systems and Laser Tissue Interaction

The intense beam of monochromatic coherent light emitted by the laser must be delivered to the target. Several different delivery methods exist, depending upon wavelength, operating power, desired spot size and accessibility of the target. The four types in common use are direct delivery, articulated arm, hollow flexible waveguide and fibre optic. At the treatment site, one of a number of applicators may be attached to the delivery system. Common applicators are lenses, sapphire contact tips, shaped or sculpted fibres, metallic or ceramic tips, diffusers and micromanipulators. (AS/NZS 4173:1994, p 20) Not all applicators can be effectively used will all lasers. The laser types and the corresponding delivery methods are shown in Table 2-8

Table 2-8 Laser delivery systems

Delivery Method	**Laser Type**
Direct Delivery	Helium-neon, diode, excimer
Articulated Arm	CO_2
Flexible Waveguide	CO_2
Fibre Optic	Argon, Nd:YAG, excimer, KTP, Krypton, helium-neon, Dye, Er:YAG, Ho:YAG, diode, alexandrite

Detailed information relating to delivery methods – direct, articulated arm, hollow flexible waveguide and fibre optic is covered in Appendix E– Delivery Methods.

The laser used for the experimental work in this thesis utilised fibre optic delivery and bipolar contact tips as the applicator. Most contact tips are made of a 'physiologically neutral synthetic sapphire crystal' (Ball, 1990 p 34). The tips have a high melting point, low thermal conductivity and low mechanical strength. The come in a variety of shapes and sizes tailored for different applications.

The advantages offered by contact tips include tactile feedback to the operator and improved cutting characteristics. The beam and hence the energy distribution can be shaped giving greater control of depth of penetration. Tips concentrate the light and this allows a reduction in the laser energy required to produce the desired effect; enhanced coagulation by direct tissue contact; less blood loss, smoke production, adjacent tissue damage and back scatter of the beam (Ball, 1990 p 34 and AS/NZS 4173:1994 p 24).

Once the laser radiation has been delivered to the target its interaction with the tissue determines its application in clinical procedures. Research has shifted towards a fundamental understanding of the interactions of light with biological tissue to allow treatment planning and to optimise laser procedures. Many studies have been conducted investigating potential tissue effects by using all types of laser systems and tissue targets. Despite virtually unlimited combinations of parameters, there are only five categories of tissue interactions that are classified and generally accepted. These interactions are photothermal, photochemical, photodisruption, photoablation and plasma-induced ablation (Niemz, 1996). This section looks at these five interactions and begins by over viewing the parameters which effect these interactions.

2.7.1 Interaction Parameters

The parameters influencing tissue interactions are the characteristics of the target tissue and the choice of laser type (wavelength) and settings (power density and exposure time). The tissue characteristics can be represented by optical and thermal properties as summarised in Table 2-9.

Table 2-9 Parameters influencing interaction mechanisms

Tissue Characteristics		**Laser Characteristics**
Optical Properties	**Thermal Properties**	
Reflection Absorption Scattering Transmission	Heat conduction (extent of vascular flow) Heat capacity	Wavelength Exposure time Power and energy density Focal spot size Mode of operation

The four specific optical properties that contribute to this interaction are the coefficients of reflection, scattering, transmission or absorption. All of which are discussed in more detail in Appendix F. Together they determine the total transmission of energy into the tissue at each wavelength. The extent of the interaction then depends on the wavelength, the exposure time, peak power, power density, mode of operation of the device and the nature of the tissue.

All these parameters share a single common datum: the characteristic energy density. This ranges from approximately 1 J/cm^2 to 1000 J/cm^2. This is surprising as the power density itself varies over 15 orders of magnitude. Thus the duration of the laser exposure is the main parameter controlling the type of tissue interaction (Niemz, 1996). Figure 2.1 maps the relationship between the major classifications of laser-tissue interactions, the characteristic energy density, exposure time and power density.

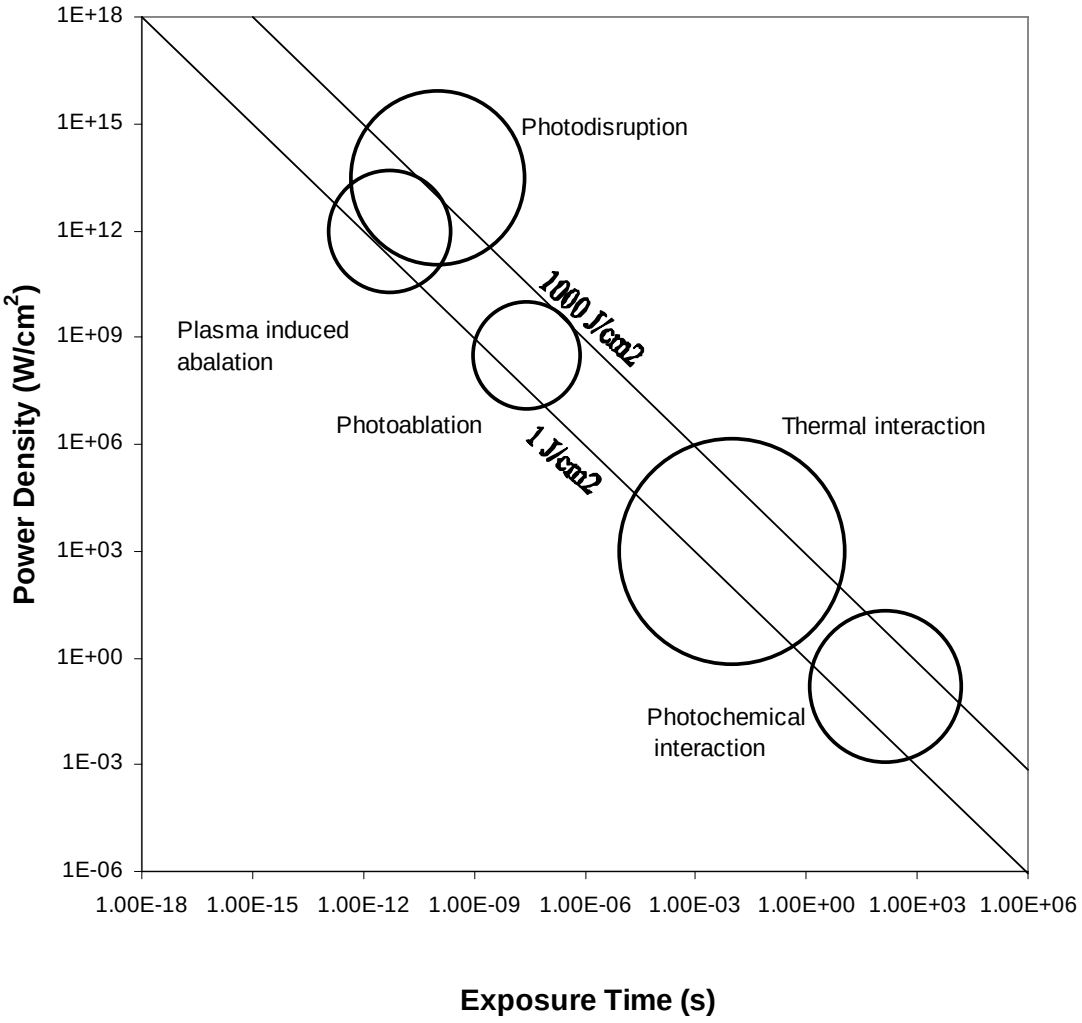


Figure 2-1 Map of laser tissue interactions

(Niemz, 1996)

The most important optical parameter is the wavelength-dependent absorption of the laser light by biomolecules e.g. in cells and tissue. The transitions of the biomolecules are equivalent to the wavelengths shorter than approximately 280 nm. In this region only a few low power lasers are available.

With the exceptions of haemoglobin in red blood cells and the pigment melanin, visible radiation is not significantly absorbed by biological objects (Frank, 1988) as highlighted by Figure 2-2 which shows the absorption characteristics for several biological tissues.

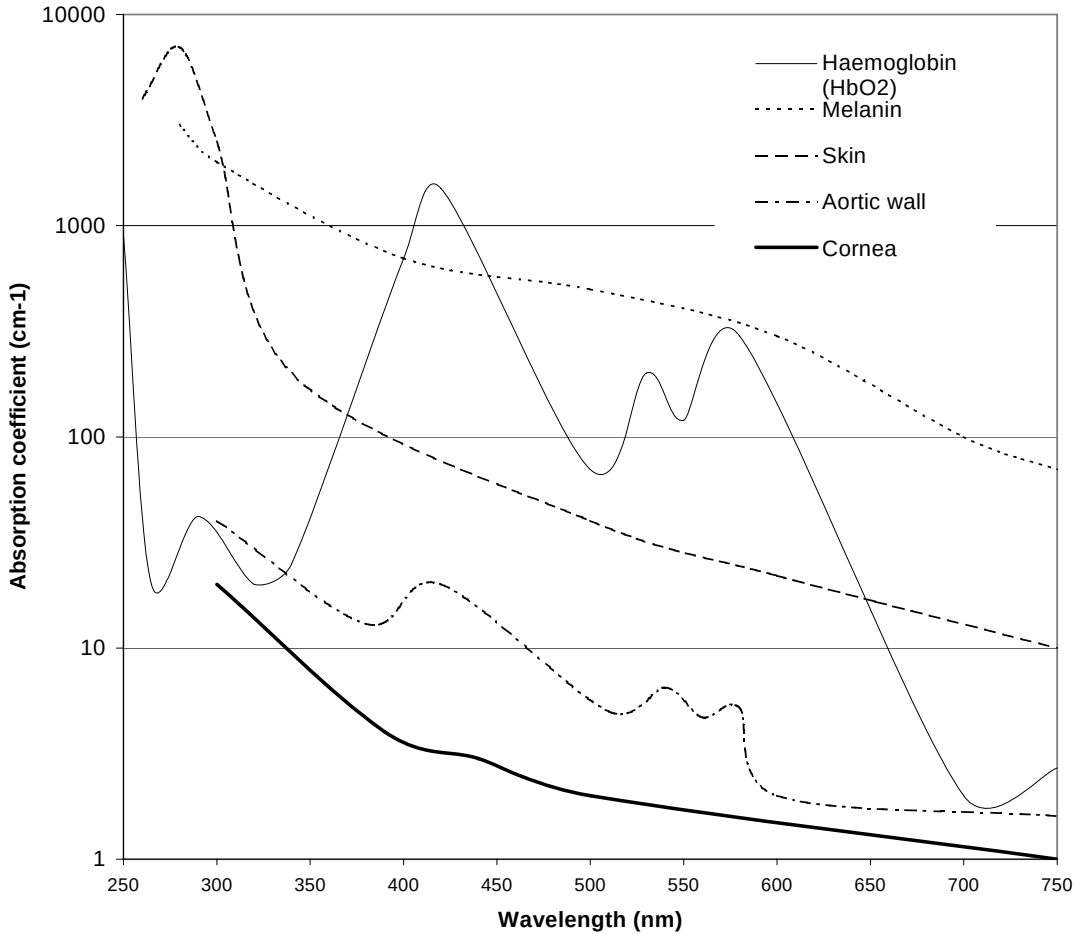


Figure 2-2 Absorption coefficients for several biological structures

Radiation in the infrared spectrum is absorbed well by most tissues due to their high water content. This leads to an extremely efficient conversion of energy and heating of tissue. A further impact on tissues is due to the process of scattering. Biological tissue is highly structured, so that a beam of light directed into it undergoes a considerable change in spatial distribution

owing to reflection, refraction and diffusion effects. Scattering occurs mainly when absorption is low (Frank, 1988).

At the characteristics wavelength of the Nd:YAG laser used in this work the main absorption characteristics of importance are melanin, haemoglobin and water. These absorption coefficients of these main constituents of skin are shown in Figure 2-3

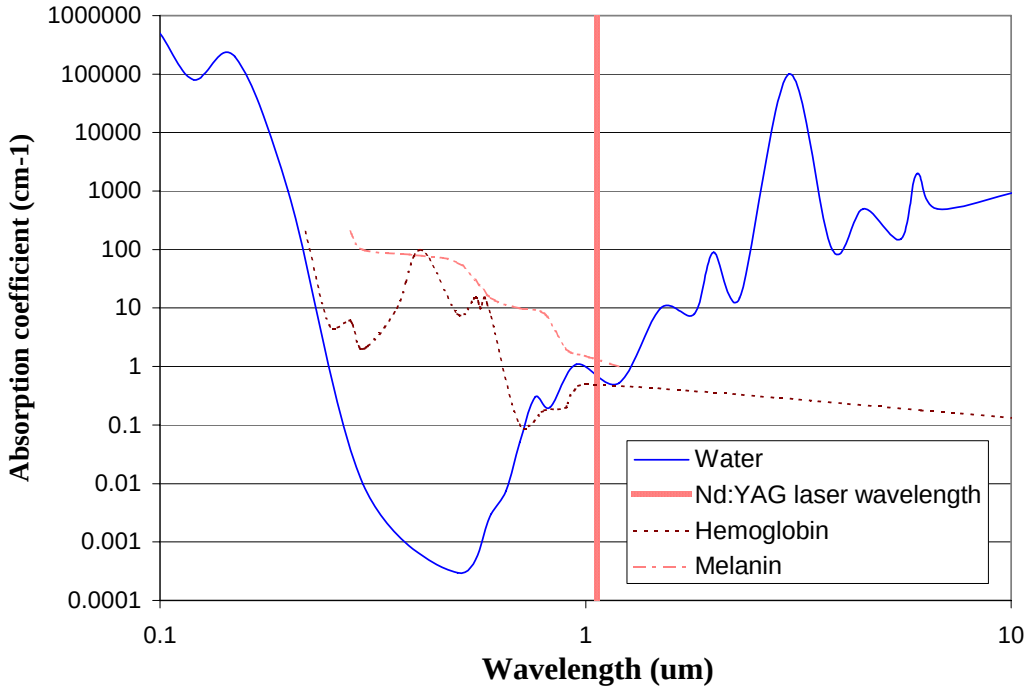


Figure 2-3 Absorption coefficient of the constituents of skin

2.7.2 Photothermal mechanisms

The thermal interaction is of primary importance for surgical applications. The degree and extent of the thermal effect depend on the optical and the thermal properties of the tissue, the geometry of the laser beam, and the energy of the incident light. These thermal effects range in a linear

fashion from protein denaturation and coagulation to vaporisation (Roenigk, 1994). Figure 2-4 schematically shows the process of thermal interactions with tissues.

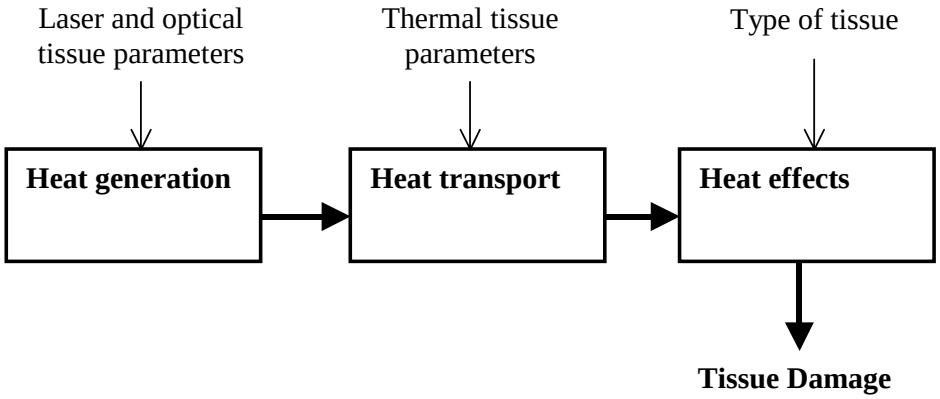


Figure 2-4 Flowchart of parameters for thermal interactions

Photothermal mechanisms are based on the conversion of light into heat as a result of absorption of laser light by tissue photoacceptors (chromophores, water and protein) and lead to denaturation or destruction of the tissue. The depth of heating depends on the penetration depth of the light into the tissue and can be induced by either CW or pulsed laser radiation. These thermal effects generally tend by be non-specific (Parrish and Deutsch, 1984). However, depending on the duration and peak value of the tissue temperature achieved, different effects like hyperthermia, coagulation, vaporisation, and melting may be seen and are discussed in the following sections.

Lasers with far and mid infra-red (IR) radiation (CO_2 and Er:YAG types) have small penetration depths due to the extremely high absorption coefficients that interstitial tissue fluids have at the wavelengths emitted. Therefore, heating effects of those lasers are very superficial. Most organic molecules absorb strongly in the ultra-violet (UV) wavelengths. Therefore, the depth of light penetration in the UV (e.g. excimer lasers) is also small, between 5 to 40 micrometers. Radiation at the near IR wavelength for example Nd:YAG, penetrate relatively deeply in the tissues. In the

visible spectrum absorption is mainly by chromophores such as haemoglobin and melanin. The wavelength of the radiation and the corresponding depth of penetration into skin is summarised in Table 2-10. It is based on Caucasian subjects with low melanin content and normal resting blood circulation (Baxter, 1994, p 80). Depending on the absorption, degree and duration of heating the thermal action can cause hyperthermia, coagulation or vaporisation as summarised in Table 2-11 while Figure 2-5 shows the locational relationship between these thermal effects.

Table 2-10 Depth of penetration into tissue for varying wavelengths

(Baxter, 1994, p 80)

Colour	**Wavlength (nm)**	**Depth of penetration (mm)**
Ultraviolet	150-380	<0.1
Violet to deep blue	390-470	approx 0.3
Blue to green	475-545	approx 0.3 – 0.5
Yellow to orange	545-600	approx 0.5 – 1.0
Red	600-650	approx 1.0 – 2
Deep red to near infrared	650-1000	2 –3
Near to mid infrared	1000-1350	3 – 5
Far infrared	1350-12000	<0.1

Table 2-11 Thermal effects of laser radiation

(Neimz, 1996 p 78)

Temperature (degree Celsius)	**Biological Effect**
37 ^{0}C	Normal
45 ^{0}C	Hyperthermia
50 ^{0}C	Reduction in enzyme activity
60 ^{0}C	Denaturation of proteins and collagen, Coagulation
80 ^{0}C	Permeabilization of membranes
100 ^{0}C	Vaporisation, Thermal decomposition (ablation)
>150 ^{0}C	Carbonisation
>300 ^{0}C	Melting

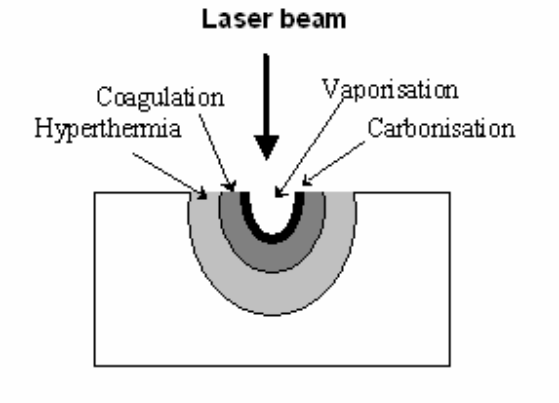


Figure 2-5 Location of thermal effects

Hyperthermia

From a normal body temperature of 37^{o}C, no measurable effects from heating are observed until approximately 42^{o}C. The first mechanism by which tissue is thermally affected can be attributed to conformational changes of molecules. These effects, accompanied by bond destruction and membrane alterations are summarised in the single term *hyperthermia*. Thus hyperthermia refers to a moderate temperature rise from approximately 41 ^{o} C to 45 ^{o} C (or even 50^{0}C) over several minutes. The hyperthermic reaction is based on the difference of thermosensitivity between normal and neoplastic cells (Mordon et al, 1987). If a state of hyperthermia lasts for several minutes, a significant percentage of the tissue will undergo `necrosis` as described by *Arrhenius' equation* (Welch and van Gemert, 1995; Person and Prahl, 1996). Beyond approximately 50^{o}C, a measurable reduction in enzyme activity is observed, resulting in a reduced energy transfer within the cell and immobility of the cell. Furthermore, certain repair mechanisms of the cells are disabled. Thereby, the fraction of surviving cells is further reduced.

It is important to recognise that as tissues change state their optical properties also change. In applications where exposure times are of the order of seconds or the power density is high the energy reaching the desired site can change as overlying tissue denatures.

The process of hyperthermia has been used as a basis for possible therapy in the treatment of neoplastic disease, as in the treatment of metastatic lever nodules by percutaneous interstitial application of (Nd:YAG) laser light, but this approach is still experimental. It is also being used in the treatment of benign prostatic hyperplasia (Dankiw, 1992).

Coagulation

Coagulation is mainly a result of denaturation of proteins and collagens which leads to necrosis of cells. The temperature that is reached during irradiation periods of about one second at 50^{o}C to 80^{o}C produces desiccation with retraction and a visible paling of the tissue. Collagen denatures at 80^{o}C. Also the tissue matrix is eliminated and the process of scarring becomes apparent.

Coagulation is used in `hemostasis`, tissue welding and induction of necrosis in small tumors. Lasers suitable for coagulation are those with a near IR wavelength radiation because of the low tissue absorption and consequently deep penetration. For vascular coagulation, lasers with radiation at visible wavelengths are also suitable because of the selective absorption by haemoglobin.

Vaporisation

Vaporisation at temperatures above 100^{o}C leads to destruction of the cellular water. The increase in temperature leads to an increase in pressure as water within the cell tries to expand in volume. This leads to localised microexplosions and is thus sometimes referred to as a thermomechanical effect. The resulting ablation is called thermal decomposition and must be distinguished from photoablation which is discussed in a following section. This vaporisation is sometimes advantageous, since the vapor generated carries away excess heat and helps to prevent any further increase in the temperature of adjacent tissue.

Vaporisation leads to a loss of substance. At a temperature higher than 100°C, the different tissue elements vaporise quickly, in about a tenth of a second. If the vaoprised zone has a comparatively large surface of some millimetres in diameter, larger tumors can be destroyed than is possible with simple coagulation. When the vaporised zone is small (100-500 micrometers) the effect of an incision is obtained.

Carbonisation and melting

If all the water molecules have been vaporised and the laser exposure is continued then an increase in temperature continues to occur. At temperatures exceeding 150^0C, carbonisation takes place which is observable by the blacking of adjacent tissue and the escape of smoke. To avoid carbonisation, the tissue is usually cooled with either water or gas. Finally beyond 300^0C, melting can occur depending on the target material.

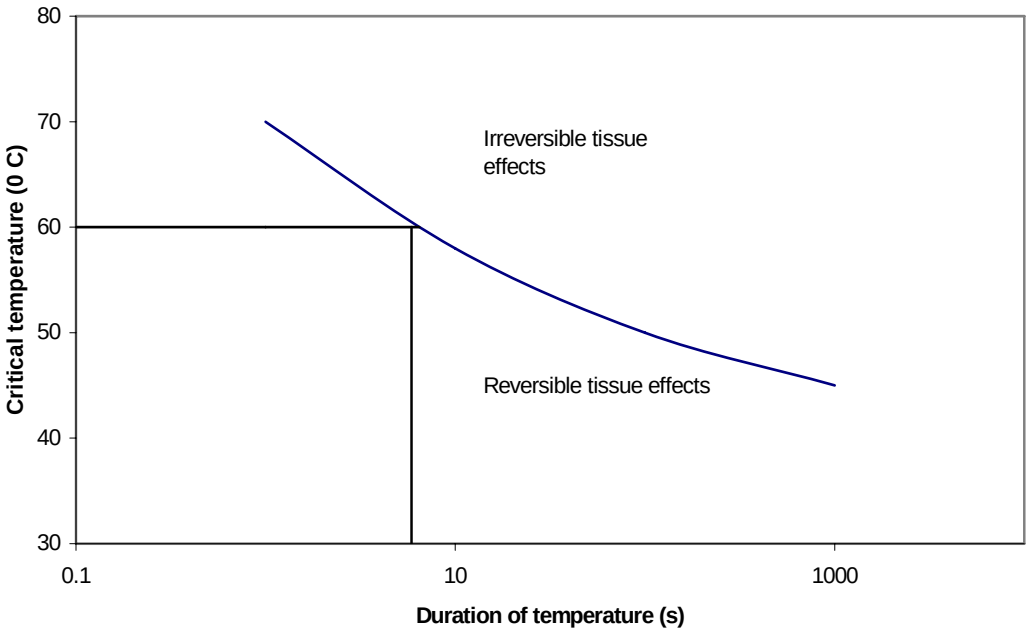


Figure 2-6 Critical temperature for the occurrence of cell necrosis

(Niemz, 1996 p 78)

2.7.3 Photochemical interaction

Photochemical interaction occurs when light stimulates or induces a chemical reaction within tissues. These reactions take place at very low power densities (typically $1W/cm^2$), and long exposure times ranging from seconds to continuous wave. Careful selection of laser parameters yields a radiation distribution inside the tissue that is determined by scattering. In most cases wavelengths in the visible range are used because of their efficiency and their high optical penetration depths (Niemz, 1996).

The interaction of laser light with photosensitising agents is referred to as photodynamic therapy (PDT) and photochemical interactions play a significant role in this process. During PDT, spectrally adapted chromophores are injected into the human body. Monochromatic irradiation can then trigger selective photochemical reactions, resulting in certain biological transformations. A chromophore compound that is capable of causing light-induced reactions in other non-absorbing molecules is called a *photosensitiser* (Neimz, 1996). Once the photosensitiser has been excited by laser radiation a diverse range of reactions take place with the end result being irreversible oxidation of essential cell structures. Thus the main ideal of a photochemical treatment is to use a chromophore receptor as a catalyst.

Currently, the prime application of photodynamic therapy is in the treatment of malignant tissues. The process involves the administration of a potent photosensitizing compound and the subsequent illumination of this compound trapped in malignant tissues by laser light.

The currently used photosensitising chemical is hematoporphyrin derivative (HpD) whose active ingredient is dihematoporphyrin ether (DHE). This compound meets the criteria for a clinically useful photosensitizer in that it is nontoxic; it is selectively retained in the tumor; it is activated by

the available penetrating light source; and finally it is photochemically efficient (Bailin et al, 1990).

The light source used most frequently for such therapy is a tuneable dye laser with its output at 630 nm. This wavelength has the dual advantages of being highly absorbed by DHE while being able to penetrate tissue well.

The laser light is delivered in a dosage of 20 to 75 joules/cm^2 via fiberoptic probes of differing sizes and shapes. The usual mechanism produces erythema in a matter of hours, followed by tumour necrosis in a few days. Higher laser light levels have been found to damage surrounding healthy skin and soft tissues (Bailin et al, 1990).

2.7.4 Photodistruption (Photoacoustical) interactions

Photodistruption is the disruption of tissue by high peak power ionising laser pulses. It is also referred to as photoacoustical or photomechanical interactions and involves the use of shock waves to fragment or disrupt tissue. Energy is concentrated in space and time to create optical breakdown or ionisation of the target medium, with the formation of plasma. (Krauss and Puliafito, 1995)

The photomechanical effect is the result of this plasma formation and *shock wave generation* induced by high power and very short laser pulses. The plasma expansion generates a spherical shock wave, leading to localised mechanical ruptures when the pressure rise is greater than the yield strength of the tissue. Photodisruption occurs when mechanical forces split the tissue. If breakdown occurs inside soft tissues or fluids, *cavitation* and *jet formation* may also take place (Niemz, 1996).

Photoacoustic effects can be achieved with a 1064 nanometer Nd:YAG laser using nanosecond or picosecond pulses, and also by a dye laser emitting light in the blue part of the spectrum with pulses of one microsecond (Dankiw, 1992).

Important applications for photoacoustical interaction have been developed in ophthalmology and urology. The Q-switch 1064 nanometers Nd:YAG laser is used for photodisrtuption of secondary cataract in ophthalmology; a pulsed dye laser (1 microsecond, 504 nanometers) is used to fragment impacted ureter stones (Danikiw, 1992).

2.7.5 Photoablative interactions

Photoabalation was first described by Srinivasan and Mayne-Banton (1982) in what they termed *ablative photodecomposition*, meaning that material is decomposed (the bonds between organic molecules in tissue are ruptured) when exposed to high intense laser irradiation (Niemz, 1996). It performs a very precise ablation of tissue which can be accurately predicted. This characteristic is utilised, for example, in making incisions on the corneas (radial keratotomy) or for the removal of atheromatous plaque from coronary arteries (Dankiw, 1992). The depth of ablation is determined by the pulse energy and the laser is pulsed in the nanosecond range. Another advantage is the lack of thermal damage to adjacent tissue either in terms of coagulation or vaporisation.

In the 1980s, the question was raised whether photoablation is based on a photochemical or a photothermal process. A review of the literature by Niemz (1996) concluded that photoablation should be considered as a seperate interaction mechanism of its own that can be distinguished from pure photochemical and thermal processes. However only an ablation caused by UV photons should be regarded as photoablation.

As photoablations can only occur by absorption of UV photons and by comparing the fluence and thermal components of laser light we can conclude that ArF excimer lasers are probably the best choice when aiming at photoablation. XeCl laser could be used for thermal decomposition, although CW laser might do a similar job. KrF laser offer both photoablative and thermal decomposition but are less useful in medical applications.

Table 2-12 summarises the laser tissue interaction shown in Figure 2-1 and discussed in this section.

Table 2-12 Summary table of laser tissue interactions

Tissue Action	Thermal Interactions	Photo-chemicial	Photo-disruption	Photoablation	Plasma Induced Ablation
Process	Achieving certain temperature which leads to the desired thermal effect	Using a photosensitiser acting as a catalyst (photo-dynamic therapy)	Fragmentation and cutting of tissue be mechanical forces	Photoablation is the breaking of molecular bonds by high energy UV photons	Ablation by ionising plasma formation
Observation	Coagulation, vaporisation, carbonisation, melting	No macroscopic observations	Plasma sparking, generation of shock waves, cavitation, jet formation	It produces a very clean, precise and predictable ablation	Very clean ablation, associated with audible report and blueish plasma sparking
Typical Lasers	CO_2, Nd:yag, Er:YAG, Ho:YAG, Argon, diode	Red dye lasers, diode lasers	Nd:YAG, Nd:YLF	excimer lasers e.g. ArF, KrF, XeCl, XeF	Nd:YAG, Nd:YLF
Typical Pulse Duration	1us to 1 minute	1s to CW	100fs to 100ns	Typical pulse durations 10 to 100 nanoseconds	100fs to 500 ps
Typical Power Densities	10 to 10^{6} W/cm^2	0.01 to 50 W/cm^2	10^{11} to 10^{16} W/cm^2	Typical power durations 10^{7} to 10^{10} W/cm^2	10^{11} to 10^{13} W/cm^2
Applications	Coagulation (tissue welding), vaporisation (cutting), melting, retinal detachment, Laser-induced interstitial thermotherapy (LITT)	Photodynamic therapy, biostimulation	Lens fragmentation, lithotripsy	Applications in ophthalmology	Refractive corneal surgery, caries therapy

3 Tissue Welding Literature Review

Tissue welding is a generic term that is also referred to as tissue fusion or vessel sealing. The process uses laser energy to activate photothermal or photochemical bonds. The advantage that lasers have over other energy sources such as radio frequency or microwave is that they provide the ability to accurately control the volume of tissue that is exposed to the activating energy (Maitland, 1999).

The exact mechanism of tissue welding is poorly understood. However, the process is now an important biomedical application of laser technology and the use of laser energy to effect a tissue weld of clinically acceptable tensile strength is now well documented (Klioze et al, 1994).

The first successful report of tissue welding seems to be that of Jain and Gorisch (1979) who studied the use of a Nd:YAG laser for the repair of small blood vessels in rats. This view is supported by Poppas et al (1993), Bass and Treat (1995) and Godkewski et al (1995).

When living tissues are exposed to extremely high irradiances in the 50,000 to 100,000 W/cm^2 range they can be incised, much like a scalpel. If the irradiances are in the moderate range of 500 to 850 W/cm^2, vaporisation of soft tissue to steam and smoke can be expected. If the tissue is exposured to low irradiances, in the 50 to 150 W/cm^2 range, coagulation will occur. This biological interaction with laser energy appears to be independent of the type of laser system used. Consequently, a number of different lasers have been employed in their low output mode of operation to attempt to close cutaneous wounds with laser energy welds (Bailin et al,1990).

The advantages of tissue welding are documented as a lack of foreign body reaction; decreased operative time; less tissue manipulation and effective union of tissues equivalent to sutured anastomoses (Gurpinar et al, 1996; Poppas et al, 1995, Poppas et al, 1993, Bass and Treat, 1995; Lewis and Urige, 1993).

Laser tissue welding is a difficult process. As the process is not well understood it is difficult to set laser parameters needed for optimum weld strength and to determine when the weld has been achieved. Energy levels and exposure times that may work well with certain tissues may not be the best for other tissues or situations (Bass and Treat, 1995).

Tissue welding[5] can be augmented with solders or biological glues, based on proteins and other compounds. These solders are applied to the surfaces to be joined and then laser light seals the solder to the tissue surfaces (Bass and Treat, 1995). The solders serve two roles. Firstly, the solders can include chromophores that are used to control the laser penetration such that it is concentrated at the fusion site. Since extrinsic chromophores are not limited to the absorption characteristics of native tissue or body fluids, solders may be tailored to selectively absorb energy that passes through normal tissue. Secondly, solders can include other biochemical constituents to improve the weld strength and or weld leakage characteristics. Typical additives include collagen, gelatinous collagen, fibrin, elastin and albumin (Matiland, 1999; Poppas et al, 1993, Cikrit et al, 1990).

[5] When additional material is added to the weld it can be known as tissue soldering which distinguished it from welding when no additional biological material is added.

Table 3-1 gives a listing of a small number of research efforts into tissue welding. It is provided to show the small range of application made to date for tissue welding. It shows that work on welding skin tissue is still at the early developmental stage.

Table 3-1 Summary of tissue welding research and clinical procedures

Type of tissue welded	**Author and year**	**Experimental (E) Medical procedure (M)**
Blood vessels (arteries and veins)	Cikrit DF et al, (1990)	E
	Kuroyanagi Y et al, (1991)	E
	Kung RTV et al, (1993)	E
	White RA et al, (1987)	E
	Tang J et al, (1994)	E
	Ruiz-Razura A et al, (1989)	E
	Flemming AFS et al, (1988)	E
	White RA et al, 1989	M
	White RA et al, (1990)	E & M
	Crew JR and Stertzer SH (1987)	M
	Kiyoshige Y et al, (1991)	E & M
	Pribil S and Powers SK (1985)	E
	Jain KK (1979)	E
Skin	DeCoste SD et al, (1992)	E
	Poppas DP et al, (1996)	E
	Garden JM et al, (1986)	E
Nerve	Ochi M et al, (1995)	E
	Menovsky T et al, (1994)	E
	Huang CT et al, (1992)	E
	Benke TA et al, (1989)	E
	Fisher DW et al, (1985)	E
Bile ducts	Oz MC et al, (1989)	
Intestine & bowel	Sauer JS et al, (1986)	E
	Vlasak J et al, (1988)	E
	Sauser JS et al, (1989)	M
Amniotic membranes	Mendoza GA et al, (1999)	E
Urinary tract	Kirsch AJ et al, (2000)	M
	Poppas DP et al, (1992)	E
Vasectomy	Shanberg A et al, (1990)	M
	Gilbert PTO and Beckert R (1989)	E
	Alefelder J et al, (1991)	E
Bone	Mourant JR et al, (1994)	E

3.1 Anastomosis

Anastomosis is the joining of two ducts or blood vessels to allow flow from one to the other. (Mosby's Medical Dictionary, 1987)

Laser tissue welding can used to close wounds or to anastomose conduits such as arteries. Anastomosis can have various forms: end-to-end, end-to-side and latero-lateral venous and arterial (Godlewski et al, 1996). In Table 3-2 provides a brief summary of researchers who have studied anatomisis using different types of lasers. It is by no means a definitive table but it does give an overview of the large number of studies completed in this area. These studies were performed with lasers of various types and techniques. In some cases the welds were completed with a dye enhancement or fibrin glue. All lasers were operated in the free-beam or non-contact mode.

Whilst operating settings, lasers and techniques have varied, all studies have reported advantages from using a laser including reduced operating time, minimal tissue damage, decreased foreign body reaction and therefore reduced inflammatory response and faster healing.

In general wound closure with the laser weld technique is the efficient when the wound edges are first approximated with either forceps, buried suture material, or stay sutures, which are removed after closure when laser surgery has been completed. The four laser systems that have been used in this fashion are the argon, CO_2, Nd:YAG at 1064 nm and the Nd:YAG at 1320 nm. It appears from initial studies that all of these different laser systems can create a functional weld (Bailin et al, 1990).

Table 3-2 Summary of types of lasers used for tissue welding

Author and year	**Laser type**
Tang J et al, 1994; Oz MC et al, 1990; Oz MC et al, 1989;	noncontact diode
Black MR et al, 1994; Cespanyi E et al, 1987; Jain KK and Gorish W, 1979	Nd:YAG
Crikit DF et al, 1990; Ruiz-Razura A et al, 1988; Quigley MR et al, 1986; Serure A et al, 1983;	CO_2
Martinot VL et al, 1994; Kuroyanagi Y et al, 1991; White RA et al, 1988; White RA et al, 1987; Godlewski S et al, 1987	Argon
Bass LS et al, 1989	THC:YAG

Successful thermal welding of soft tissues has been reported but primarily for vascular structures and not skin. Jain (1980) published a paper on a sutureless technique for microvascular anastomosis using the Nd:YAG laser. He used typical laser parameters of 18 Watts of power, focal spot sizes of 0.3mm and single exposure durations of 0.1 s. This method proved to be considerably faster the conventional suture techniques; did not damage the endothelium of the vessel and could be performed on relatively small and/or deeply located blood vessels.

The optimum temperature for welding is nonspecific and the exact mechanisms are not well understood but are believed to rely on heat-induced alterations in collagen of the vessel (Neimz, 1996; Welch and van Gemert, 1993; Bass et al, 1992). Conflicting values have been published for different types of vascular anastomoses irradiated at a variety of wavelengths: 80-120^{o}C for CO_2 induced microvascular anastomosis (Badeau et al, 1986 and Kopchock et al, 1988); 45-50^{o}C for argon laser treated arterial-vein anastomosis under saline irrigation (Kopchock et al, 1988) and 90-140^{o}C measured by 3mm-diameter thermistors placed on the surfaces of aortic disks irradiated at 1.06 μm (Jenkins et al, 1988). Clearly the effective welding temperatures are

inconsistent and dependent on wavelength, tissue type, irradiation parameters and boundary conditions. Definitive temperatures are close to impossible to achieve since thermal denaturation of proteins is a rate process driven by energy and not temperature thresholds. (Welch and van Gemert, 1993)

However despite the assumption by Welch and van Gemert (1993) that the process is determined by energy and not temperature considerable time and effort have been spent by many researchers determining optimum temperature for effective welds. This research has focused on ultimate tissue temperature and not the net energy focused on the tissue by a particular laser.

Martinot (1994) proposed that with the use of the argon laser in carotid end-to-end anastomosis in rats could be achieved by different functions of laser parameters, (mode and power) and time. He concluded that an average temperature of 77^0C at the site of the laser impacts on the vessel wall could produce reliable anastomosis.

3.2 Tissue welding (skin)

"Laser techniques for joining tissue, in combination with other surgical technologies, will be a hall mark of surgery in the next century" (Bass and Treat, 1995). Such is the optimism held by researchers for this technology. In reality however much work needs to be done, both in research and education of the medical profession. The conservative nature of the medical profession generally means that change is slow and methodical and the advances must represent significant improvements over current technology and practices before they are adopted. Surgical wound closure has progressed in this manner from the days when a wound was seared with a hot iron and dressed with hot oil, to the sophisticated art of today's plastic surgeon. Laser tissue welding may represent further progress as an important adjunct to current methods of wound closure (Dew, 1986).

The conventional technique for closure of cutaneous wounds entails approximation of the wound edges by sutures or staples. In the majority of cases this leads to a satisfactory seal and scar formation. However the application of sutures in some cases can lead to a foreign body reaction, development of keloid or hypertrophic scar and infections (Abergel et al, 1986; Bass and Treat, 1992). In addition, sutures require a complex series of movements (Bass and Treat, 1992; Seki, 1988) and are "skill intensive". The end result relies on surgeon assessment of suture tension and spacing (Bass and Treat, 1992). Nevertheless sutures can be adapted to almost any tissue condition, are inexpensive reliable and readily available (Bass and Treat, 1992).

Other types of mechanical closures such as staples or clips have a more uniform result, due to the use of the stapling device itself, however they also have limitations due to the inability to adapt for tissue thickness and friability (Bass and Treat, 1992).

The first reports of skin laser welding were reported by Garden et al (1986) and Abergel et al (1986) using CO_2 and Nd:YAG free beam lasers respectively. Subsequent work has shown laser welding of skin offers several advantages including a minimal inflammatory response, minimal manipulation and tissue trauma, and consequently reduced scar formation and improved cosmetic results (Reali et al, 1994 and Godlewski et al, 1995).

Despite these advantages welding of cutaneous laser has not progressed beyond the experimental results to clinical works. In fact whilst there has been significant work done in the area of anastomosis little has been reported in the area of skin welding. Fortunately much of the anastomosis work is relevant and can be applied to skin closure. Below in Table 3-3 provides a summary of some of the authors publishing papers relating specifically on skin welding. It should be noted that all of this work is with free beam lasers as apposed to contact tip applicators.

Table 3-3 Brief summary of cutaneous tissue welding

Author and year	Laser type	Summary
DeCoste SD et al, 1992	Alexandrite	Dye-enhanced; guinea pig skin
Poppas DP et al, 1996	1320 nm Nd:YAG	Temperature controlled; pig skin
Abergel RP et al, 1986	Nd:YAG	Free beam; hairless mice
Garden JM et al, 1986	CO_2	Comparative tensile strength studies of the lasers to the suture and staple for wound closure
Reali UM et al, 1994	Diode	Dye-enhanced; rat skin
Murray LW et al, 1989	Argon	Crosslinking of extracellular matrix proteins; a possible mechanism of argon laser welding
Wider TM et al, 1991	Argon	Dye enhanced laser welding and fibrinogen
Dew DK et al, 1991	1.32 microns Nd:YAG	Software driven medical laser system
Chuck RS et al, 1989	Diode	Dye-enhanced
Lemole GM et al, 1991		Evaluation of collagen as a component in the thermally-induced weld

A detailed review of the papers Table 3-3 indicates that several issues relevant to welding studies are in need of discussion:

- optimisation of the type of laser used for a specific application
- required power density dependent on tissue type and thickness
- operating mode of laser including optimal spot size, exposure time and power level
- delivery system e.g. fiber optic, articulated arm, microscope mounted

It was noted from the review that the selective absorption of different laser wavelengths by tissue chromophores determines the net temperature of the tissue as affected by both vertical and horizontal heat diffusion, the penetration depth and hence the depth of tissue necrosis or hyperthermia (temperature affected without killing cells). Even when the absorption characteristics of a particular laser are well established, the net effect of irradiation may change from patient to patient, procedure to procedure. For example the CO_2 laser is highly absorbed by water and blood. If both the amount of surface and interstitial water and blood vary than the net effect of the laser irradiation will change.

3.3 Mechanisms of tissue welding

Successful thermal welding of soft tissue has been reported primarily for vascular structures however the mechanisms of tissue welding still remain unclear and this poor understanding of the process inhibits the selection of ideal laser parameters (Welch and van Gemert, 1995; Bass et al, 1992). Many authors consider that activation of collagen molecules plays a crucial role in the thermally induced weld (Reali et al, 1994 and Lemole et al, 1991). The laser energy is believed to weld tissue by changing the triple helix structure of collagen and yielding noncovalent bonds (Mendoza et al, 1999; Schober et al, 1986).

4 Neodymium: Yttrium Aluminium Garnet (Nd:YAG) Laser

"The successful operation of the first working laser by Theodore Mainman in 1960 was like the key to a locked chest full of other types of lasers. Only six years later working prototypes of gas, liquid, solid and semiconductor lasers had been constructed. By the end of that decade, hundreds of materials had been found capable of laser action." (Fisher, 1988)

4.1 Nd:YAG Operation

The Nd:YAG laser consists of a solid crystal of yttrium aluminium garnet. It is doped with a rare earth, neodymium that actually produces the laser light energy when exposed to bright flash lamps. There are three frequencies, which can be produced by this crystal, but the usual wavelength is 1064nm. This frequency is located in the near infrared region of the electromagnetic spectrum. The laser operation can be modified to produce the frequency doubled Nd:YAG with a wavelength of 532 nm (see KTP laser in Appendix D). The latest Nd:YAG laser produces a wavelength of 1318nm.

The Nd:YAG laser can be operated either in the continuous (CW) mode or in a pulsed mode. The CW laser system has special power requirements and some systems require external plumbing connections to provide a constant flow of cool water. Other systems are air cooled with an internal closed water supply cooled by a fan.

4.2 History

Snitzer (1961) reported achieving laser action in barium crown glass doped with neodymium ions. This was followed by Geusis et al (1964) who announced laser action in other neodymium-doped garnets.

Frank (1988) reported the first continuous wave Nd:YAG laser working on the 1064 nm wavelength as being introduced in the medical field in 1973. One assumes this report refers to the introduction of the first medical specific system, as there had been earlier reports of the Nd:YAG laser use in (medical) experimental work. A significant application of the Nd:YAG laser to surgery was reported by Kiefhaber (1977) to control massive gastrointestinal bleeding in humans (Fisher, 1987).

Early in the development of medical Nd:YAG laser it become apparent that laser radiation is only as useful as the available accessory instruments. These are required by the surgeon to take full advantage of the efficient properties of the laser wavelength. In 1984 sapphire contact probes were introduced by Joffe and Diakuzono (1985). These tips placed in contact with the target tissue permit precise cutting of soft tissue with excellent hemostasis of transacted vessels but without the extensive thermal damage characteristic of a free beam Nd:YAG laser (Fisher, 1987). The developments of the Nd:YAG laser particularly relating to medical areas are summarised in Table 4-1 on page 47.

Today the Nd:YAG laser is a standard instrument in general laser surgery. It has gained popularity because of its excellent coagulative effects on tissue. In addition its ability to be transmitted through available quartz fibers makes the Nd:YAG laser ideal for endoscopic surgery (Rebeiz et al, 1990). Its applications extend to bronchology, gastroenterology, dermatology, gynaecology, ophthalmology, neurosurgery, urology and vascular surgery (Fisher, 1988).

The Nd:YAG laser has good coagulative properties but its thermal effect extends beyond the immediate visible ablative effects. There can be extensive thermal damage beyond the area of actual tissue ablation which may cause a danger to the patient e.g. perforation of the tracheobronchial tree when removing tumours (Rebeiz et al, 1990). The introduction of a pulsed

wave (PW) mode to the Nd:YAG laser may permit better control and causing less damage to surrounding tissue (Rebeiz et al, 1990).

With the use of nonlinear optical materials, such as potassium phosphate, it may be possible in the near future to build a frequency-quadrupled Nd:YAG laser to produce a wavelength of 266 nm for the precise surgery now being done experimentally with various excimer lasers. Perhaps the most important application of all will be biostimulation, where Abergel et al (1984) have demonstrated the ability of rays at 1064 nm to lyse collagen in keloids. Uses of this kind may well overshadow the purely surgical application of the Nd:YAG laser in the future.

Table 4-1 History of development of Nd:YAG medical laser

Year	Author	Application
1965	Minton et al, (1965)	Use of ruby and pulsed Nd:YAG laser on the Cloudman S-91 mouse melanoma
1971	Fidler JP (1971)	100W Nd:YAG laser with Nath fiber to incise canine levers
1971	Mussigang H and Katsaros W (1971)	Studied the effects of 25 W Nd:YAG laser on excised tissues
1973	Commercial Development	Introduction of the mediLas 100W Nd:YAG laser by MBB-Medizintechnik (MBB-AT)
1977	Kiefhaber P et al, (1977)	Control massive gastrointestinal bleeding in humans
1978	Hofstetter A et al, (1978)	Coagulation of tumors of bladder wall using an optical fiber to transmit the laser energy
1979	Toty L et al, (1979)	Endoscopic treatment of tracheobronchial lesions
1980	Aron-Rosa D and Griesemann JC (1980)	Mode-locked picosecond pulsed Nd:YAG laser to cut vitreous strands
1980	Fankhauser F (1983)	Q-switched, nanosecond-pulsed Nd:YAG laser for ophthalmic applications
1983	Dumon JF and Meric B (1983)	Palliative treatment of obstructing malignant tumors of the airway
1984	Joffe S and Daikuzono N (1985)	Introduction of the first sapphire tips for quartz optical fibers permitting contact laser applications
1985	Joffe S and Daikuzono N (1985)	Artificial sapphire probe for contact photocoagulation and tissue vaporization with the Nd:YAG laser.
1986	Abergel RP et al, (1986)	Reported experimental work using the Nd:YAG laser welding of skin (in mice)
1992	Nishiwaki Y et al, (1992)	Introduction of preliminary results using Nd:YAG laser bipolar dissector.

4.3 Effect of Nd:YAG laser light on tissue

The Nd:YAG laser emits light in the near-infrared range at 1064 and 1318 nm. The primary mechanism of action on biological tissue is dependent upon the conversion of this radiant optical

energy into thermal energy. This conversion of light energy into thermal energy depends upon absorption and scattering of the beam with in the tissue. Once the light is absorbed, it delivers energy to the tissue, and the tissue's reaction depends on the intensity and exposure time of the light. A less intense, longer pulse will cause rapid heating, or a photothermal effect. Lower intensities applied for longer durations with cause a photochemical change, either by a slow transfer of energy as heat or by a specific chemical reaction. In actual practice, all of these interactions coexist, although by selecting the proper wavelength, intensity, and pulse duration, the desired effect can be maximized. The one major exception to this rule is the mechanism of the Q-switched or mode-locked laser, which is capable of producing short bursts (10^{-9} to 10^{-12} seconds) of high power levels, inducing mechanical as well as thermal damage to the tissue.

Halldorsson et al (1981) established a low absorption coefficient (α) of 0.11 and a scattering coefficient (β) of 9.89 per centimetre of tissue traversed by the beam. If absorption alone were responsible for dissipating the energy of the beam, penetration would reach approximately 9 cm, as was proved with a sample of water. However, skin is not solely composed of water. The complex and non-homogeneous three-dimensional array of proteins within the cells and intercellular matrix causes a high degree of scattering within a relatively short distance below the surface of the tissue, making this the more significant parameter (Brackett, 1988). Scattering converts the incident coherent column of photons into a diffuse oblate spheroid of uniform distribution of the radiation in the tissue (Halldorsson et al, 1981; Mackenzie and Carruth, 1984; Frank, 1988).

The tissue volume that is covered by the laser light is heated slowly around the point of impingement, followed by a deep, slowly progressing coagulation. This results in delayed destruction of the tissue with no noticeable structural damage and coagulation down to 6mm in

depth (Frank, 1988). This deep coagulative effect can seal both blood and lymph vessels (Frank, 1988; Frank et al, 1985). Blood vessels are sealed by the combined effect of shrinkage and uniform coagulation of tissue. Arteries of up to 2mm and veins of up to 3 mm in diameter can be closed rapidly and reliably (Beck et al, 1979).

The type and amount of tissue damage inflicted by the Nd:YAG laser is also dependent on surface cooling. This can be achieved by gas or in the case of the 1064 nm laser, water. When the surface is cooled a deep, drop-shaped necrosis develops, the surface of the tissue hardly being injured. The first effect is blanching on the surface of the skin. This blanching limits the expanding volume of coagulation due to backscatter. If carbonisation occurs on the surface, absorption increases and the tissue is vaporised. If the surface of the tissue is cooled with water the blanching effect is delayed, carbonisation avoided, with the result that deep coagulation occurs (Landthaler et al, 1984).

In pulsed laser systems the pulse structure and duration have been shown to be important parameters in controlling thermal damage. The pulse duration and repetition frequency affect the thermal relaxation time of the tissue and the thermal recovery time respectively (Sanders and Reinisch, 2000).

4.4 Noncontact Delivery Systems

Since the first introduction of the first medical laser systems, many companies have followed suit. To date there are approximately 14 different companies marketing Nd:YAG laser systems ranging in maximum power from the 40 W to 120 W.

All these instruments have essentially the same hardware components - the laser head, which accommodates all the optical elements of the Nd:YAG laser: crystal, pump lamps, and resonator

mirrors, as well as a pilot laser within the visible spectrum. The pilot light is produced by a helium-neon laser in the milliwatt range, and is concentric with the main invisible beam of the Nd:YAG laser. Both are transmitted to the operative fields via a flexible quartz fibre light guide. The pilot light marks the target point of the therapeutic beam.

Commercially available quartz optical fibres with a strong Teflon covering (fibre diameter 0.2 - 0.6 mm) fulfill all the requirements relating to mechanical stability and high flexibility. Laser light is introduced at the proximal end by means of a lens system whose focal point is adjusted to the fibre core diameter. The transmission efficiency of these fibre systems is 90%. The exit divergence ranges of 6^0 to 24^0 and can be compensated by adjusting the irradiation distance or by the use of appropriate lenses.

Gas-cooled or liquid-flushed light guide systems are available. The gas is introduced into the light guide at the optical coupling. A suitable nozzle at the distal end provides efficient irrigation and cooling. The rinsing solution is either introduced in the same way or more often directly connected to the endoscope of handpiece which is used together with the light guide. The fibre tip can easily be repaired if damaged. Systems from 1 to 2.6 mm in diameter are available.

4.5 Contact Delivery Systems

The Nd:YAG laser has several drawbacks when used in the free beam (non-contact) mode. These include poor cutting, excessive backscatter, inaccuracy, and excessive tissue damage. However on the positive side, the laser is perhaps the best thermal coagulator and has good penetration of the target tissue.

In 1985 a Japanese company, SLT Laser, introduced contact laser probes of a "specially selected, physiologically neutral synthetic sapphire crystal with great mechanical strength, low thermal

conductivity, and a high melting temperature" (Daikuzono, 1988; Diakuzono and Joffe, 1985). These probes offered a completely new method of delivering the Nd:YAG energy to tissue, and overcome the limitations stated above. The probes used in direct contact with tissue, allow precisely controlled manipulations and restores the tactile feedback that was lost in conventional laser techniques.

A conventional, noncontact YAG laser delivery systems emits a diverging beam of gradually increasing size and diminishing power density. Approximately 30 to 40% of the beam energy can be lost to backscatter, and a portion of the rest is expended on non-targeted healthy tissue due to inaccurate focusing and beam scattering. A contact probe creates a well-defined localised region of high-power density right at the tip of the probe, which is placed precisely against the target tissue as shown schematically in Figure 4-1. The problem of focusing is completely eliminated. Spot size and power density are under accurate and precise control. Energy loss to backscatter is cut to less than 5% and the overall laser output needed to achieve a given therapeutic effect is 75 to 90% lower that would be needed in a non-contact procedure. Since less total energy is delivered to the site, damage to healthy neighbouring tissue is greatly reduced (Daikuzono, 1988). Table 4-2 compares the required power and related power density of contact and contact mode of the Nd:YAG laser.

The contact probes come in various shapes, each with an individual energy distribution pattern. The user determines the precise spot size and power density by selecting the appropriate probe and laser power, and so control the shape and volume of thermal effect. This is not possible with any non-contact laser or other thermal techniques when used on cutaneous tissue.

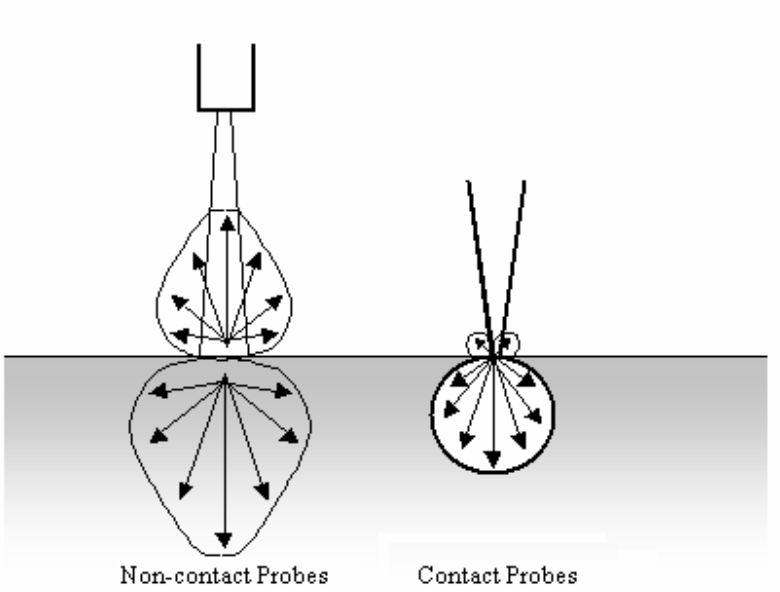


Figure 4-1 Nd:YAG laser energy distribution in tissue and loss through backscatter

(Daikuzono, 1987, p 21)

Table 4-2 Comparison of contact and non-contact power

(Daikuzono, 1987, p 20)

Laser power (W) required to achieve given power density at the tissue surface		
Contact Nd:YAG	**Noncontact Nd:YAG**	**Power density (W/cm^2)**
1	12	700
5	62	3500
10	124	7000
15	185	10500
20	247	14000
25	309	17500

Additional advantages of the contact probes include significantly less blood loss, ability to control the balance between cutting and coagulation and reduced smoke generation as summarised in Table 4-3 and Table 4-4. The most significant difference is in the extent of tissue necrosis around the target site. The non-contact resection causes up to 3 mm of lateral necrosis, depending on the laser power. The contact procedure does not cause necrosis of more than 0.5 mm under any circumstances (Daikuzono, 1987).

As with noncontact probes a coolant delivery system of gas or water can bring several advantages to clinical applications e.g. cooling adjacent normal tissue and reducing fumes.

Table 4-3 Comparison of laser features with Nd:YAG contact and non-contact modes

(Daikuzono, 1988, p 21)

			Nd:YAG	
Feature	**CO_2**	**Argon**	**Noncontact**	**Contact**
Beam easily deliverable to any part of the body	No	Yes	Yes	Yes
Beam transmissible through fluids	No	Yes	Yes	Yes
Convenience in providing any required power density	Moderate	Low	Moderate	High
Precisely controllable focal point	No	No	No	Yes
Degree of damage to healthy tissue	Low	Moderate	High	Low
Smoke generation	High	Moderate	High	Low
Tactile feedback	No	No	No	Yes
Laser power requirements	High	High	High	Low
Laser maintenance requirements	Moderate	High	Low	Low
Delivery system maintenance requirements	Moderate	High	High	Low

Table 4-4 Characteristics of contact and noncontact Nd:YAG

(Joffe and Dwyer, 1987, p 40)

Characteristic	**Noncontact**	**Contact**
Power levels		
Coagulation	60-100 W	10 W
Vaporisation	70-100 W	8-15 W
Blood loss	High	Low
Smoke generation	High	Low
Width and depth of thermal damage	3-5mm	0.2-1.0mm
Energy loss to backscatter	30-40%	5%
Tactile feedback	No	Yes
Pain to patient	Moderate	Low

Nishiwaki et al (1992) released preliminary results of work completed with a new type of probe - a Nd:YAG laser bipolar dissector. In this type of probe the laser energy is delivered medially from the distal 7mm of each probe which is produced from synthetic sapphire. The radiation profile from the probes is shown schematically in Figure 4-2. Compressing the arms of the forceps can approximate the distal ends of the probes. These probes have the ability to coagulate larger vessels than the single contact probes. Nishiwaki was successful in sealing veins up to 5mm and arteries up to 2mm without re-bleeding.

This thesis investigates the use of these bipolar contact probes in cutaneous tissue welding.

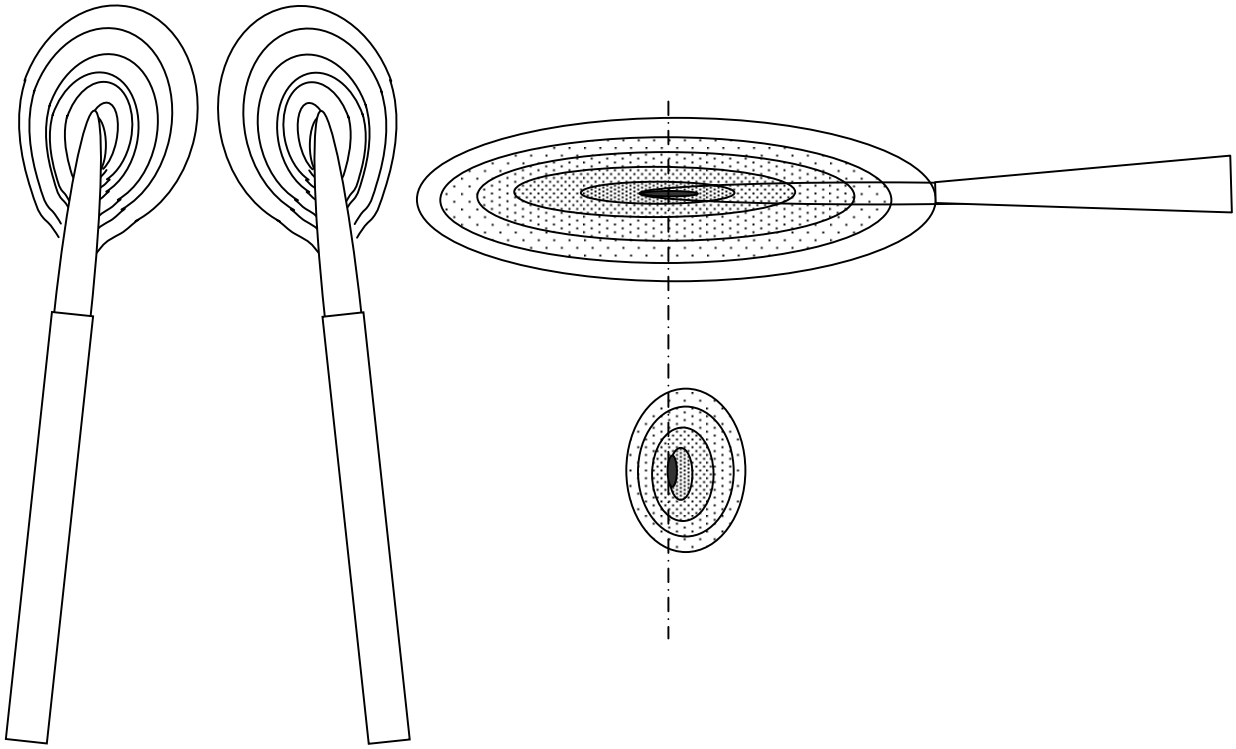


Figure 4-2 Radiation from bipolar probes

(Nishiwaki et al, 1992)

4.6 Parameters relevant to tissue welding using Nd:YAG contact probes

There is no known published information relating to the use of Nd:YAG contact probes to weld cutaneous tissue. Required information includes the absorption and scattering coefficients for *skin* and the power and mode settings to achieve the required laser tissue interaction.

The laser – tissue interaction required is coagulation. The skin tissue had to be heated sufficiently to obtain a viable weld, but with out appreciable cell necrosis. Progressive necrosis of severely damaged cells over several days would weaken the weld before sufficient healing could take place to naturally bind the wound.

Daikuzono (1987) investigated many parameters of the Nd:YAG contact system and their dependence on the power setting of the laser. Resection of rat liver was done in both clinical and experimental studies. At 10 – 20 watts cell necrosis was approximately 0.2mm at day 0 and virtually non-existent at 15 days (Figure 4-3); smoke production was nil to very small; and blood loss was moderate to nil (Figure 4-4). Daikuzono did not specify the mode of operation of the laser i.e. CW or pulsed mode. The lack of necrosis at day 15 implies more rapid healing following contact surgery.

Significant differences exit between Daikuzono's investigation and the work reported in this thesis with respect to the target tissue and the dominant laser- tissue interaction.

In this thesis the target tissue is skin while Daikuzono used liver tissue. It has already been established that Nd:YAG wavelength is absorbed mainly by haemoglobin and to a smaller extent melanin. Rat liver has a much higher haemoglobin content than skin and virtually no melanin. Consequently the absorption characteristics and the corresponding tissue's reaction to the light will be different to that recorded by Daikuzono (1987). Secondly the required tissue reaction for welding is coagulation only. In resection the dominant effect is ablation with some coagulation in surrounding tissue to seal blood vessels which have been cut.

It is concluded from this review that while specific testing is needed to get the parameters for skin and a coagulation effect, a power setting of 10 watts, CW mode and a contact time of between 1 and 5 seconds would provide a safe starting point for this investigation.

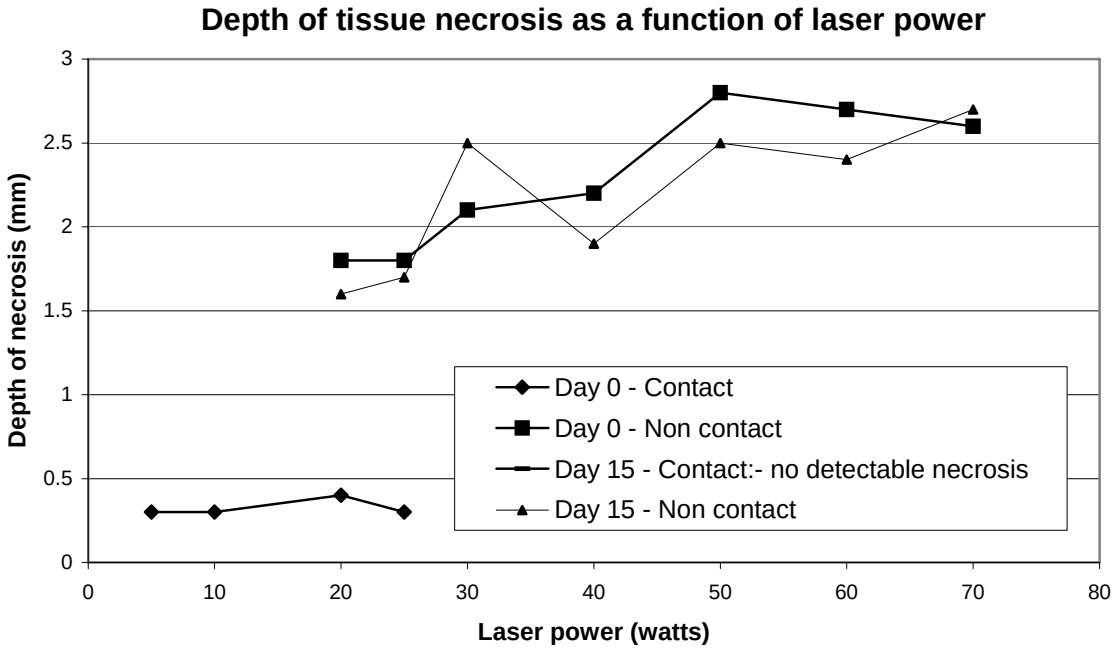


Figure 4-3 Depth of tissue necrosis as a function of laser power

(Daikuzono, 1987, p 23)

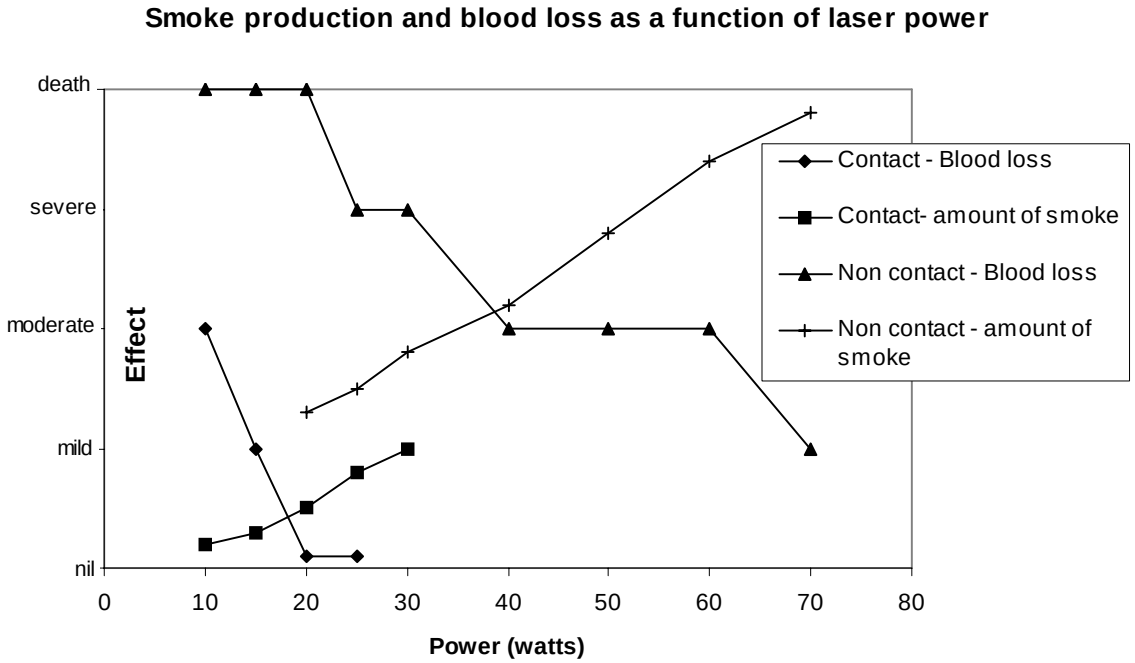


Figure 4-4 Laser power relationship for smoke production and blood loss

(Daikuzono, 1987, p 24)

5 Methodology

The experimental objectives of this thesis are to:

1. Obtain tensile strength data for uninjured rat skin for use as a baseline when comparing the healing rate of lasered and sutured skin.
2. Evaluate the performance of Nd:YAG bipolar contact laser in welding cutaneous tissue in rats as measured by healing rates relative to sutured skin.

Based on the review of existing information on lasers, a standard Nd:YAG medical contact laser with bipolar contact probes was selected to test the welding performance on a number of rats with uniform skin characteristics. Access to the laser was obtained through Dr Geoffrey Buckham and Dr Ross Harrington at St Andrews Hospital, Toowoomba.

Inbred Wistar rats were made available to the project by the University of Southern Queensland (USQ) Animal House. The USQ Animal Ethics Committee (approval number 97REA020) approved the research program. The animals were cared for in accordance with USQ guidelines and control.

The experimental program was designed in three phases as summarised in the Table 5-1 below.

Table 5-1 Summary of experimental stages

Phase 1	Investigation of base line tensile strength of rat skin.
Phase 2	Determination of power and mode settings for optimal tissue temperature to achieve a satisfactory weld.
Phase 3	Comparison of strength of welded wound to sutured wound, at regular intervals of time.

5.1 Ethics

The number of animals available for this investigation was limited by the USQ Ethics Committee. The guidelines produced by this committee state *"experiments involving animals will be permitted only if they are essential for the investigation of matters associated with …. the advancement of medical science"* and *"experiments should employ no more animals than are (statistically) necessary."* (Guidelines For The Preparation Of Applications For Ethic Clearance, sections 4 and 9). Appendix G- 1 contains the complete guidelines whilst Appendix G- 2 contains the application to the Ethics committee and Appendix G- 3 contains the subsequent approval.

Further clearances were acquired from St Andrews Hospital, Toowoomba and Queensland Government (Queensland Health). These clearances were necessary because the experiment was undertaken at a private Toowoomba hospital. Such research is outside their licensing agreement and requires special permission from the Queensland Department of Health. Appendix G- 4 and 5 contains copy of approval from St Andrews Hospital and Queensland Health.

5.2 Experimental Design

5.2.1 Number of Animals

The number of animals available to this investigation was limited as described in the previous section. The limitation imposed classified this experiment as an initial investigation to establish the viability of the technique. Should the technique, including type of laser prove to be successful, the committee indicated that a larger study with greater sample numbers might be acceptable to validate the procedure.

The numbers allowed by the USQ Ethics Committee for each stage of the program were:

Phase 1 - 5 rats to test base line tensile strength of skin vs orientation and position of test sample

Phase 2 - 2 rats to determine power and time settings of laser for optimum weld strength

Phase 3 - 15 to 20 rats to compare weld strength with that of sutured skin

5.2.2 Justification of animal usage

It was necessary to minimise the number of experimental variables in order to maximise the information obtained from the permitted number of animals. The rats were matched for age, sex and general health. The strength of healthy skin and welded skin was determined in one orientation and at one elongation speed (strain rate).

The adopted experimental procedure was the established as follows.

Phase 1 - Base Line Samples

Haut (1989) studied the effect of the orientation and location on the strength of 36 rat skins. Variables included location (head and back), orientation (parallel and perpendicular to the spine) and elongation speed (2) in the tensile tests. Based on their published results, it was decided to analyse the tensile strength of the skin at set locations keeping a constant orientation (parallel to the spine only) and elongation speed. A small test specimen (micro-tensile test die ASTM D-1708) was chosen to enable a larger number of test specimens to be taken from the one animal.

The initial plan was to sample only the area of skin which would be sutured or lasered, thus giving an indication of the strength of uninjured skin and hence a target strength to determine when the skin was fully healed. This would result in a total of thirty (30) sample data points: six (6) samples per rat (three samples from each side) by 5 rats as shown in Figure 5-1(a). However

very little literature was found with quantitative information on the tensile strength of rat skin and so it was decided to utilise the full skin and take as many samples as possible. This modification to the procedure would maximise the experimental information obtained from the limited number of rats. The final plan for tensile testing allowed for up to twenty-one (21) samples per rat, giving a significantly larger sample number. See Figure 5-1(b).

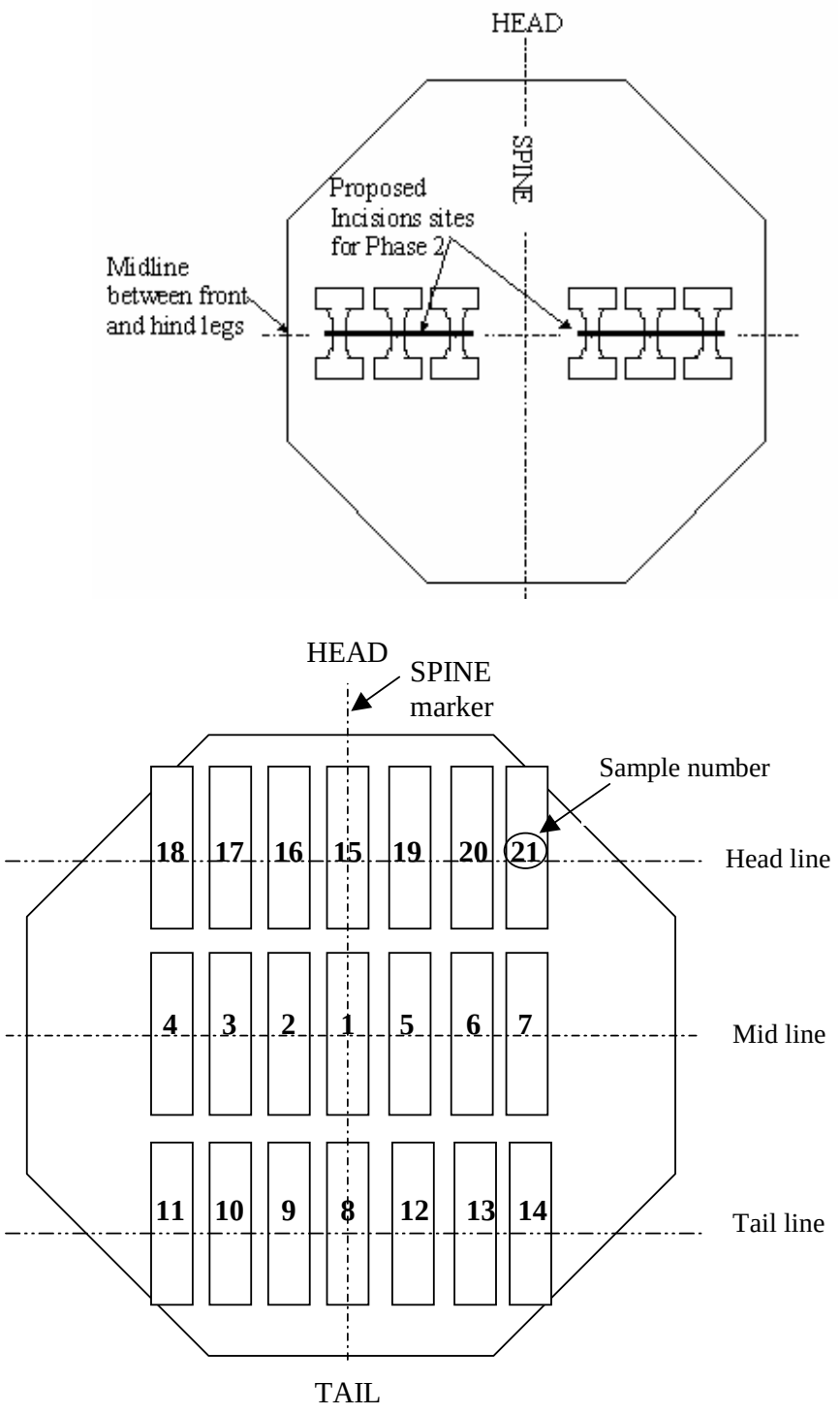


Figure 5-1 (a) Initial plan for tensile testing (b) Final plan for testing

Phase 2 Laser Settings

Chapter 4 shows that optimum temperature for welding is non-specific and the exact mechanisms of joining the skin tissue are not well understood. It is believed to rely on heat-induced alterations in collagen of the tissue (Neimz, 1996; Welch and Van Gemert, 1993). Collagen denatures at approximately 80^0C and at this point the tissue matrix is eliminated and the process of scarring becomes apparent. The principle of coagulation which is used in hemostasis, tissue welding and induction necrosis in small tumors takes place at a temperature somewhere between 50 and 80 ^{0}C. The exact temperature is not known. The tissue temperature achieved by laser radiation depends on the absorption coefficients of tissue photoacceptors (chromophors, water and protein) and its interaction with the specific wavelength of the laser and the mode of operation of the laser (continuous or pulsed). There is currently no data for the mode and power settings of a **contact** laser and the corresponding tissue temperature. In this phase different laser settings, the corresponding temperature in skin and effectiveness of the welding process were to be evaluated.

The laser was set to a variety of power settings and tested in both the CW and pulsed mode. The temperature induced in surrounding tissue from the contact probes was monitored both by infrared thermometer and a small bead thermistor inserted in the tissue approximately 1 to 1.5 mm from and parallel to the wound. The bead thermistors used were R-T curve match thermistors. With a thermal response time of 0.1 second and calibrated to +- 0.2^0C over a temperature range of -80^0 C to $+150^0$ C (RS catalogue number 232-4538). The infrared thermometer has a spot size of between 1 and 10 mm depending on distance from target.

Phase 3 - Strength of Welded Skin

Current medical literature favours small experimental sample sizes with an implied assumption of small variances in the total population. Francis et al, (1996) investigated the use of CO_2 and

argon lasers for tendon repair. The study design used 40 Spraque Dawley rats divided in to 4 groups of 10; Taylor et al, (1997) used a group of 36 rats divided in 3 groups of 12.

This project adopted slightly larger sample sizes with a number of samples taken from each animal. Animals were to be sacrificed over a seven (7) week period at a rate of two (2) animals per week. Six samples per animal were planned including three samples of laser treatment and three sutured treatment. It was intended to obtain a measure of both in-rat and between rat variation from these samples.

The rats were to be transported to the laser installation at St Andrews Hospital, anaesthetised, incised at the nominated sites and then laser welded and sutured. Each rat was to be photographed immediately after treatment and then at regular intervals during the healing process.

5.3 General Care of Experimental Animals

Rats were housed in single cages in the USQ Animal House both before the experiment and during the healing stage. Two trained technicians responsible for the Animal House oversaw general care (food, water and cleaning). Animals had access to a continuous supply of food and water and the temperature of the room was controlled at a constant 20^{o}C. The rats were identified by permanent marker on the tail and ear indicating batch and rat number e.g. 1-4 indicates batch number 1, rat number 4. The cage was also labelled with this information in addition to other information including sex, date of birth and initial body weight.

Post operatively the rats were transported back to the USQ Animal House and isolated from the general population of the animal house. They were monitored half hourly until they had fully

regained consciousness. In addition a 'mush' of food pellets, water and glucose was supplied on the floor of the cage until the animals had recovered sufficiently to reach for food and water which was held on top of the cage.

Rats were not given any medication for pain relief but were monitored four times daily in the early stages following the procedure to ensure they were not unduly stressed and were not worrying their wounds by gnawing or scratching etc. Any animal that was found to be suffering unduly or had wounds which reopened were either euthanaised or sutured, as appropriate. This was in accordance with the USQ Guidelines for the care of laboratory animals.

5.4 Tensile testing of rat skin

Haut RC (1989) published the only comparable tests found in the literature on strength of rat skin and this work was used as a basis for the experimental approach in this project. Haut's work indicated a typical tensile response of between 4 and 6 MPa for slow speed failure experiments (extension rate of 30% per second) dependant on orientation of the test specimen.

5.4.1 Test Equipment

The adopted equipment for this phase of the experiment included:

- A Microtensile test die manufactured to ASTM D-1708 standards (equivalent to ISO 12086-2:1955) as shown in Figure 5-2
- Screw action clamps
- Servo-controlled hydraulic test machine fitted with a 250N load cell
- High speed video camera

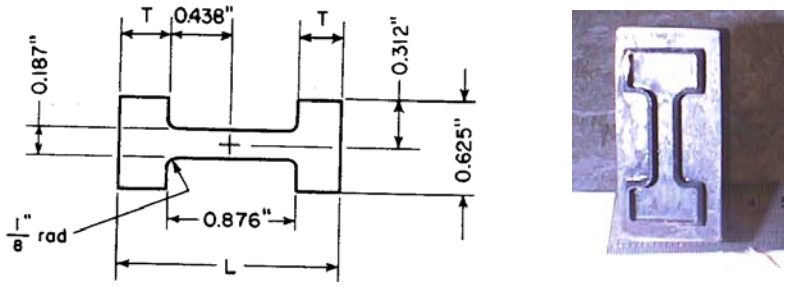


Figure 5-2 Microtensile test die manufactured to ASTM D-1708 standards

The clamps chosen were Houndsfield HT50; described as "Ultra light vice grip" by the manufacturer. The grip consists of flat rubber coated jaw faces controlled by a screw action. They are manufactured for a range of testing applications including sheet materials, paper, plastic films and waxed paper. The specifications for the clamps are listed in Table 5-2.

Table 5-2 Specifications for mechanical clamps (HT50)

Maximum capacity	1.5kN
Minimum load cell	50N
Gripping length	18mm
Maximum sample width	75mm
Maximum sample thickness	5mm
Weight each	380g
Length each	75mm
Temperature limits	-70^{o}C to +200^{o}C

These clamps were mounted in a servo-controlled hydraulic test machine (Hounsfield Universal Testing Machine Model H5K-S) which was fitted with a 250N Tensile load cell. Both the load cell and testing machine were calibrated and certified to NATA standards. The outputs of the

machine were ported to a HP Laser jet printer in the form of a force vs elongation graph for each specimen.

Table 5-3 contains the specifications for the Houndsfield Universal Testing Machine.

The 250N load cell was calibrated and was "found to indicate better than +/-0.5% deviation from the true applied force" (Certificate of Calibration, 1999). A copy of the Certificate of Calibration is found in Appendix H).

Table 5-3 Specifications for Houndsfield Universal Testing Machine
(Hounsfield Test Equipment System Reference Manual, 1999, page 4)

Force Measuring System	Full bridge strain gauge load cell of Z-Beam construction for use in tension or compressions. Interchangeable load cells are available and include self-identifying connectors.
Force Measuring Resolution	1/320000 of load cell capacity of the full range
Force Accuracy	+/- 0.5% of the indicated load
Force Sampling Rate	200 Hz
Extension Measuring System	Precision encoder operated directly from the crosshead driving ballscrew for readout of extension (position)
Extension Resolution	0.001mm of the full crosshead travel
Extension Accuracy	+/-0.01mm
Speed accuracy	+/- 0.05% of the set speed

5.4.2 Test Procedures

Initial base line values for the tensile strength at different positions on the body were first evaluated. This was done to determine the extent of variation in the strength of the skin at different body locations and between rats. Test specimens of skin were punched parallel to the spine and tested to failure with a constant elongation rate of 30% per second of the initial length of the test sample. A plot of Force (newtons, N) against elongation (mm) was printed for each test specimen.

Nineteen rats (fourteen females and five males) were selected to determine these baseline parameters of maximum tensile force and elongation. Initially it was planned to use only five aged matched female rats but a supply of extra female and male rats became available from other science experiments (dissection studies only). These extra rats were used to increase sample numbers and give an indication of the similarity (or otherwise) between the base line tensile strength of the skin of males and females and the variation with age.

The rats were euthanased by gassing in carbon dioxide, as per USQ Guildlines. The skin was then shaved and marked with the position of the spine, orientation (left, right, head and tail), batch and rat number was marked using a fine permanent marker. The skin was then excised using the `blunt dissection` method. This process involves the freeing the skin from the underlying muscle by breaking the loose layers of connective tissue. Using scissors and scalpel to cut through the skin along the midline of the stomach around the neck and tail and around the limbs mostly achieved excision of the skin. A closed pair of round end scissors was then inserted under the skin and opened to break the bands of connective tissue and free it from the muscle with minimum damage to the skin or the muscle and blood vessels. Extreme care was taken

during the dissection process to avoid damaging or applying any stress to the skin and wound sites. Figure 5-3 shows the blunt dissection process being used on one of the rats.

The excised skin was kept moist with a phosphate buffered solution as indicated by Haut RC (1989) and testing was completed within two hours of sacrifice. Test specimens were punched from the skin using the micro-tensile test die at the locations indicated in Figure 5-4. The exact number punched from each skin varied from the schematic diagram. Some animals had a large fatty deposit in the area of samples 18, 21, 11 and 14 or a variation in size of the animal both of which made it impossible to punch or reliably test these samples.

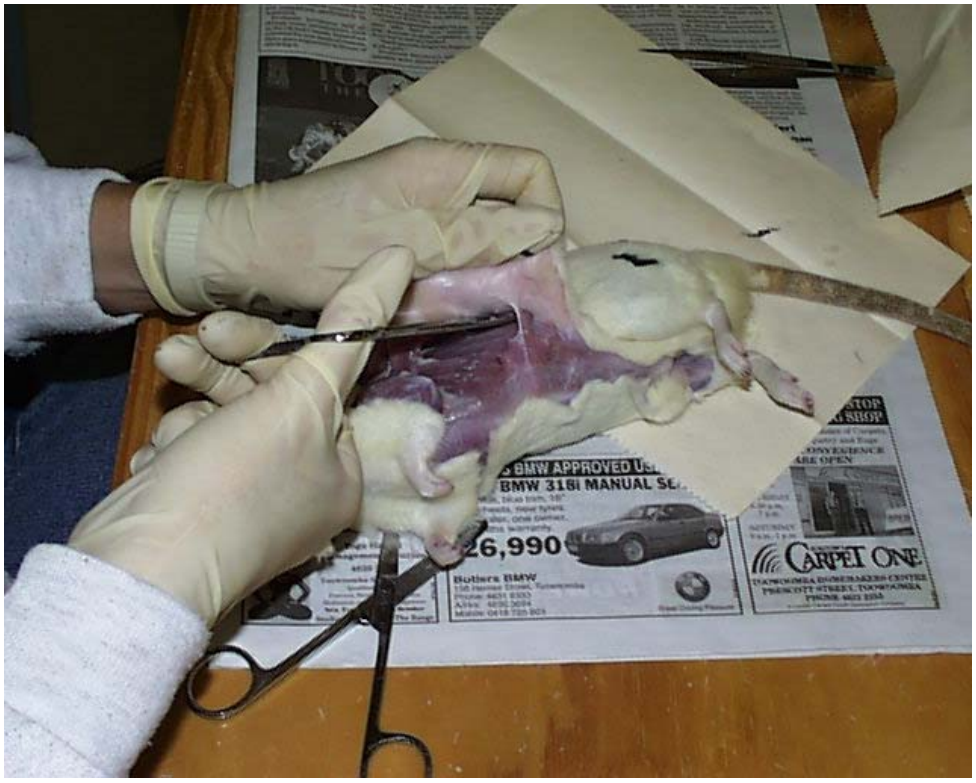


Figure 5-3 Blunt dissection process

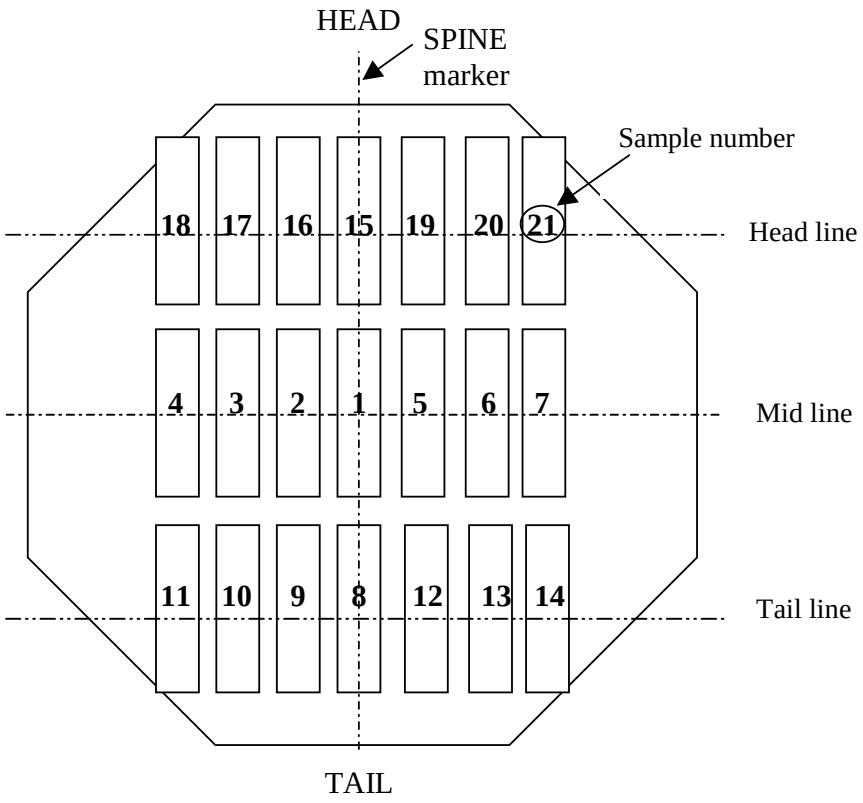


Figure 5-4 Test position layout

In an initial test it was found that any stretching of the skin whilst punching (e.g. pinning to dissection plate) resulted in a "shrink back" of the punched test specimen as shown in Figure 5-5. Unless the test specimens were consistent in size the percentage elongation at failure could not be accurately determined from the samples. Therefore the skin was not pulled taut by any means before or during punching to avoid this shrink back of the specimen. The specimens were continually moistened with the phosphate-buffered saline solution before testing to avoid dehydration.

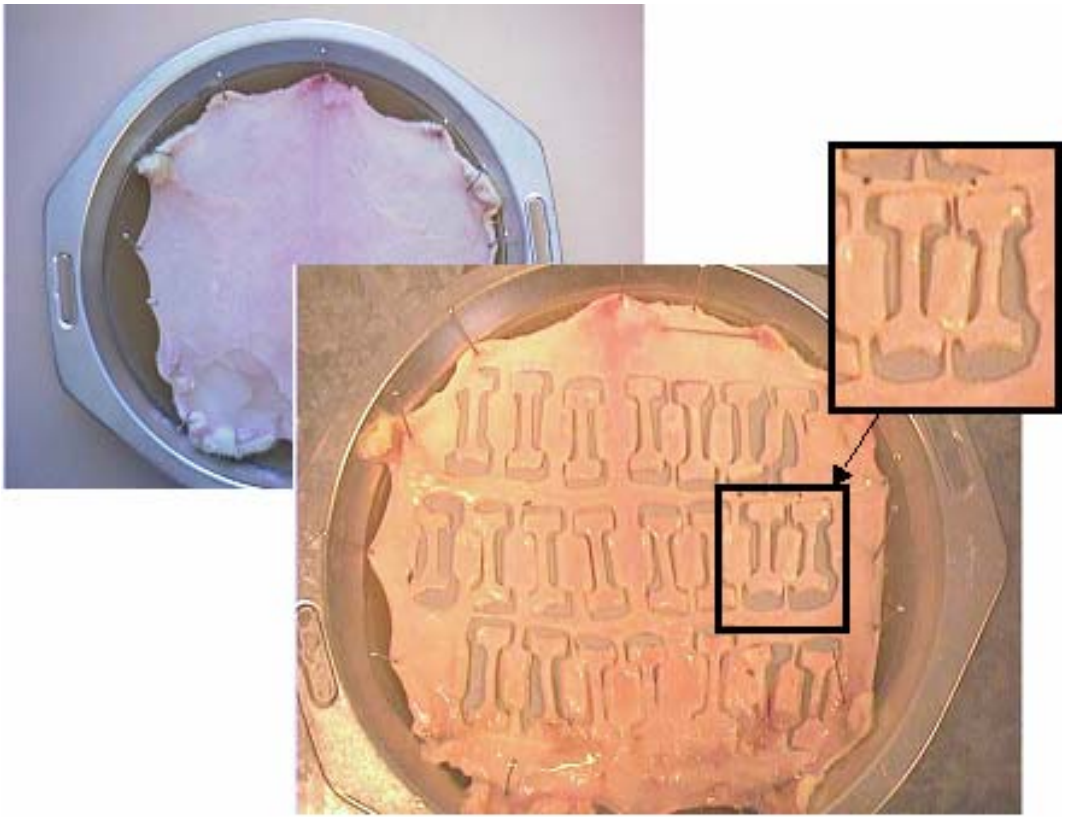


Figure 5-5 Test specimen showing "shrink back "

The test specimens were placed between to small pieces of sandpaper, secured and mounted in the clamps as shown in Figure 5-6. The sandpaper prevented slippage of the specimen in the mechanical clamps which otherwise tended to occur because of the lack of friction on moist fatty surfaces on the skin.

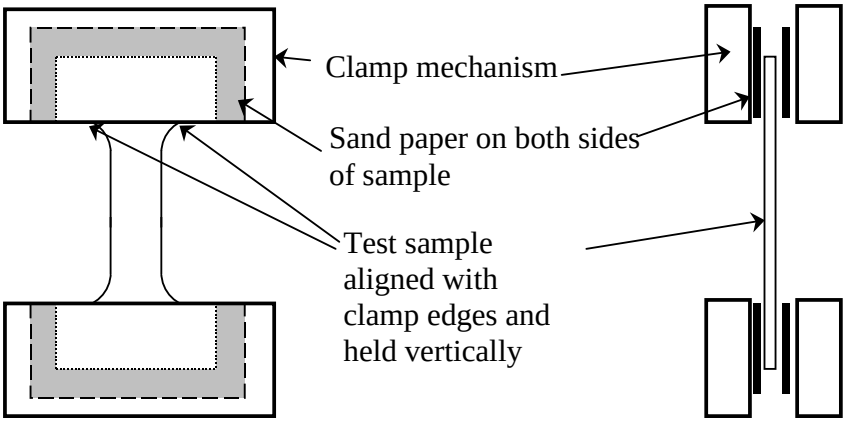


Figure 5-6 Mounting of test sample in clamps

Specimens were stretched to failure at a rate of 1.14 mm/second (equivalent to 30 percent /second of initial specimen length). This rate was constant for all tensile tests because the rate of strain has a significant effect on the maximum stress at failure (Haut, 1989). The elongation of the specimen at failure was recorded. It was expected that the elongation would be a constant percentage of the initial length.

In all tests, break force (N) and elongation (mm) were recorded and plotted. Laboratory notes were maintained to record any unusual features of the tests, such as failure at the top or bottom of the specimen, slippage from clamps, unusual amounts of fat or connective tissue present on sample and unusual failure characteristics.

Statistical analysis of the breakforce and elongation was undertaken to determine the difference in tensile strength of uninjured skin for male and female rats and the effect of the position of the test specimen. Results are presented the in next chapter.

5.5 Laser welding versus suturing

This phase of the experimental program required the laser welding and suturing of twenty female rats matched in age, weight and general health. The rats were between 287 and 320 days old and weighed between 270 and 280 grams. All were considered to be in good health by the animal house experts.

Rats were anaesthetised with Ketamine (Ketamav, 50-100 mg/kg) and Xylazine (Rompun, 10-20 mg/kg) as a combined intraperitoneal injection of 20mg/kg. They were injected just prior to the procedure and a whisker reaction check was done to ensure they were fully anaesthetised before beginning the experiement. At this point in time a more specific health check of each rat was undertaken including an inspection of teeth and gums and any signs of external injuries to the animal (cuts, scratches, bites etc) which could provide sites for infection and hence a delay in healing.

The rats were shaved around the site of the procedure and marked (ear and tail for batch and rat number; spine position and suture and welding side). No swabbing of the wound site was undertaken to eliminate the chance of excessive heating of the skin due to absorption of the laser.

Two incisions were made perpendicular to the spine mid way between the front and hind legs with a #10 scalpel blade. These incisions were 50 mm in length and 10 mm from the spine as shown in Figure 5-7. One incision was sutured, whilst the other was welded using the Nd:YAG laser and bipolar contact probes.

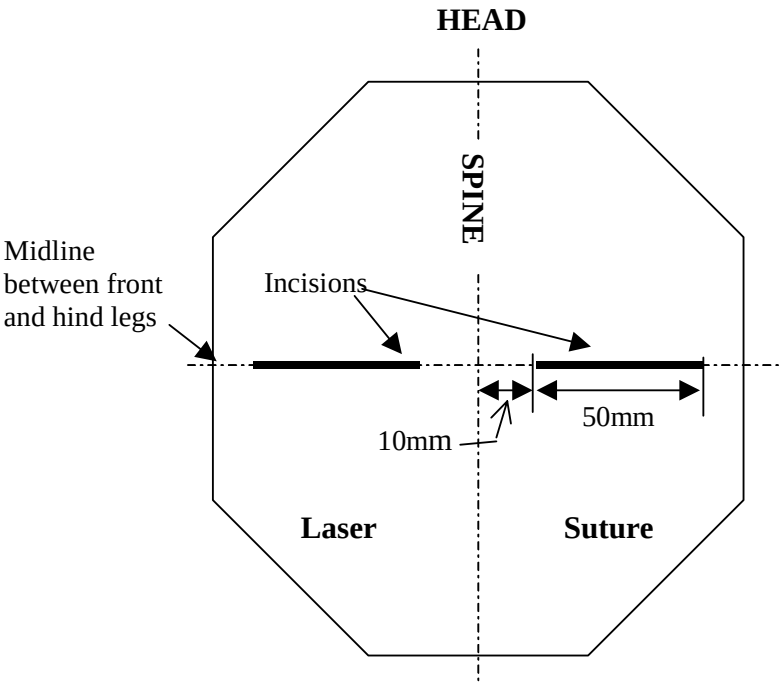


Figure 5-7 Position of incisions

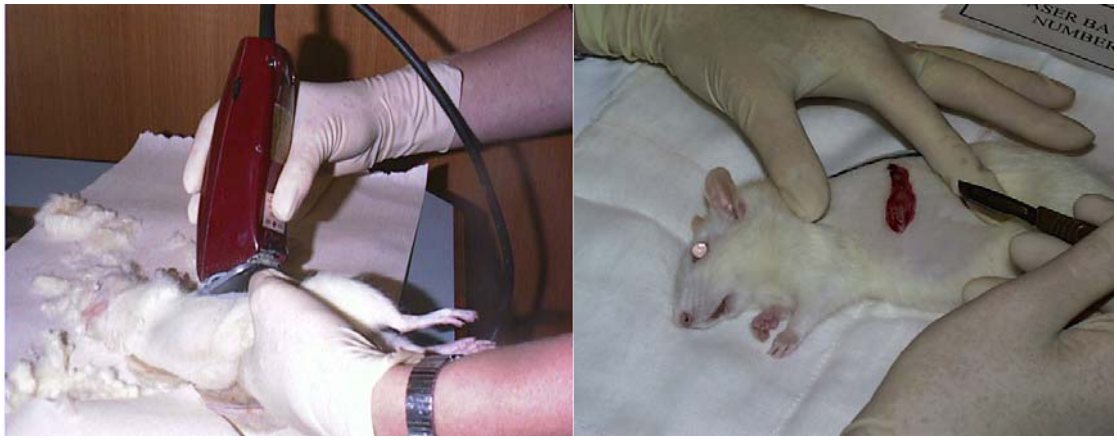


Figure 5-8 (a) Preparation of wound site (b) incision on rat

5.5.1 Suturing

The incision to be sutured was closed using standard suturing techniques and equipment. Sutures were placed approximately 8 mm apart on advice from animal care experts. Care was taken not to over tension the suture and so cause 'puckering' of the skin. The same number of sutures were inserted in each rat and no disinfectant was applied to the wound. The rat was returned to the Animal House and the wound left to heal naturally.

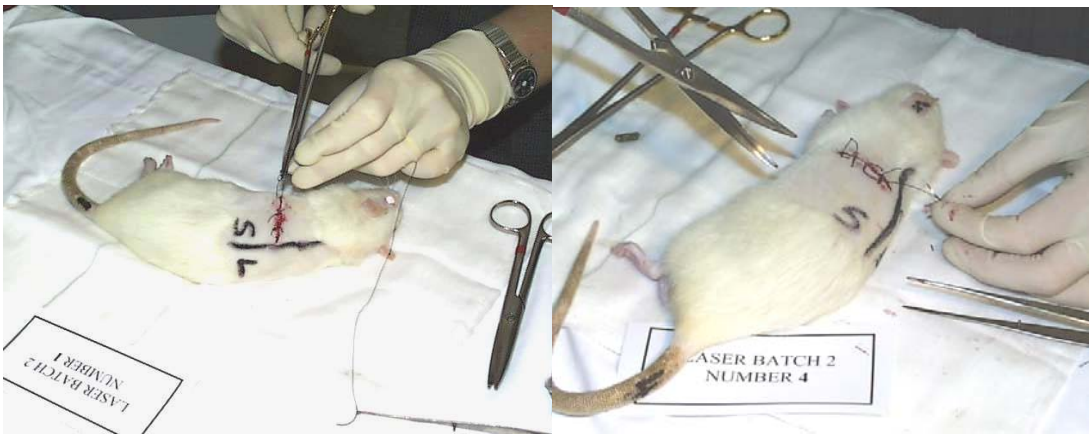


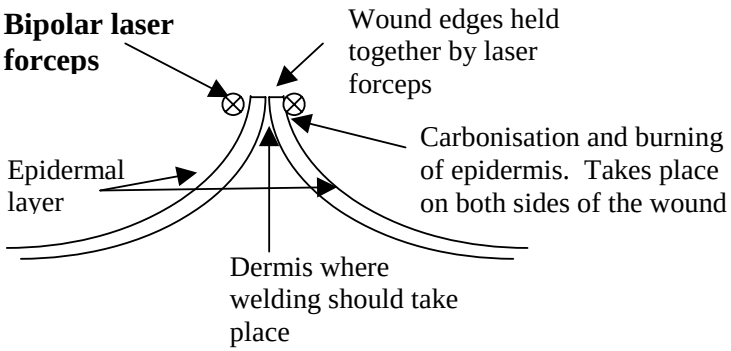
Figure 5-9 Suturing of rats

5.5.2 Laser welding

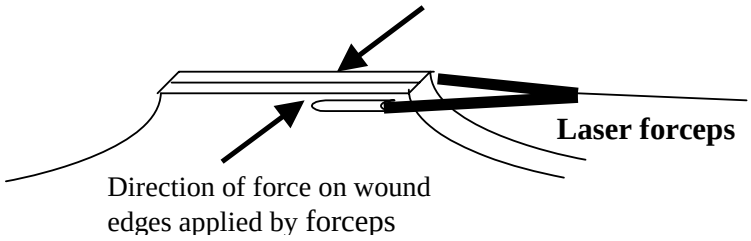
Laser welding of the skin incision was completed using a SLT[6] Nd:YAG laser with bipolar contact probes at a setting of 10 W continuous power. In the preliminary study (Phase 2) this setting was found to induce a temperature of approximately 55^{o}C in the surrounding tissue when the probes where heated for approximately three seconds. Studies by Welch et al (1989) concluded that this temperature was ideal to induce melting of the collagen fibres sufficient to allow welding to take place.

The initial welding procedure planned to use the forceps mechanism of the probes to both hold the edges of the wound together whilst applying the laser action as shown in Figure 5-10. However this proved unworkable due to the thickness of the rat skin. The heat generated by the laser over a period of time caused burning and carbonisation of the epidermis and insufficient penetration of the heat to the dermis to induce welding.

[6] The use of company names and models of laser are for descriptive purposes only and do not imply an endorsement of any particular product.



Cross sectional view of wound and laser forceps showing carbonisation of epidermis.



Longitudinal section of laser forceps on wound causing carbonisation of epidermis

Figure 5-10 Initial welding technique (a and b)

The successful technique developed and employed on twenty experimental animals used a two stage welding process and two pairs of forceps as shown in Figure 5-11. One pair of forceps held the wound together whilst the laser forceps were used to weld the inner layers of the skin. In the second stage of the process the bipolar probes were held together and placed over the top layers of skin whilst the wound was still held in position by the forceps. The laser was removed and the wound allowed to cool (thermal relaxation) for a few seconds before the first forceps were removed. Figure 5-13 and Figure 5-14 show the welding process and the sutured and lasered wounds during one day after the procedure.

One resulting disadvantage of this technique was that it resulted in *spot* welds on the wound rather than a continuous welding process which occurs with welding or soldering in the free-beam mode. The spot welds were kept as close together as physically possible. The length of the tips of the forceps was 7 mm and thus the spots welds could be describes as 'pseudo-continuous'. However the contoured shape of the ends means that even pressure along the tips could not be guaranteed or tested.

The carbonisation of hair during the welding technique was a small but potentially significant problem. Smoke extract would be recommended if future large scale studies were attempted.

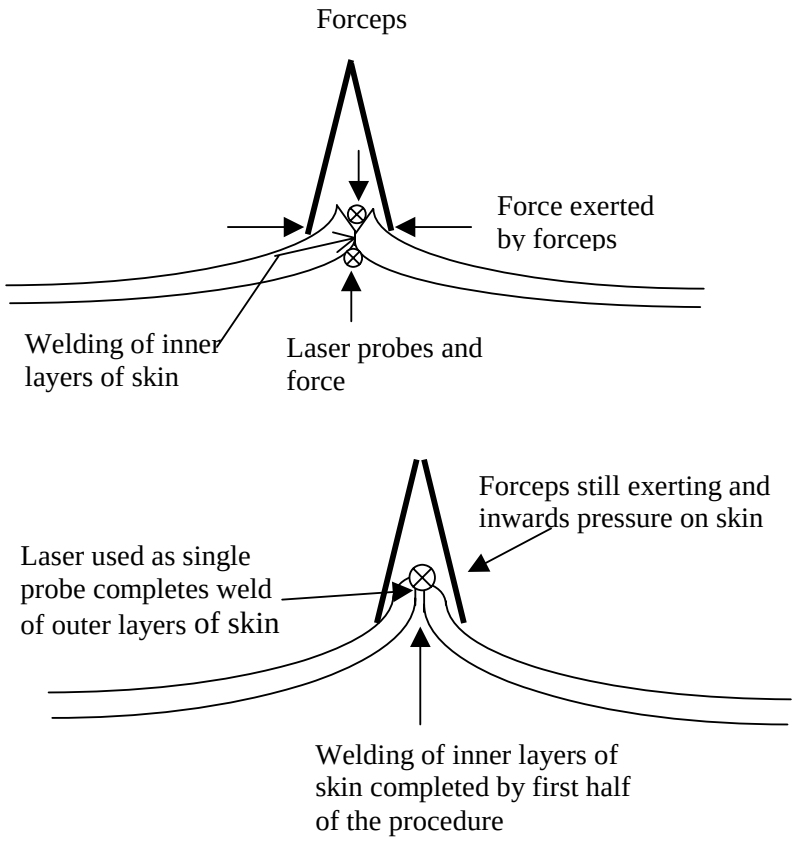


Figure 5-11 Final welding technique

Figure 5-12 Tissue welding using bipolar Nd:YAG laser and appropriate safety glasses

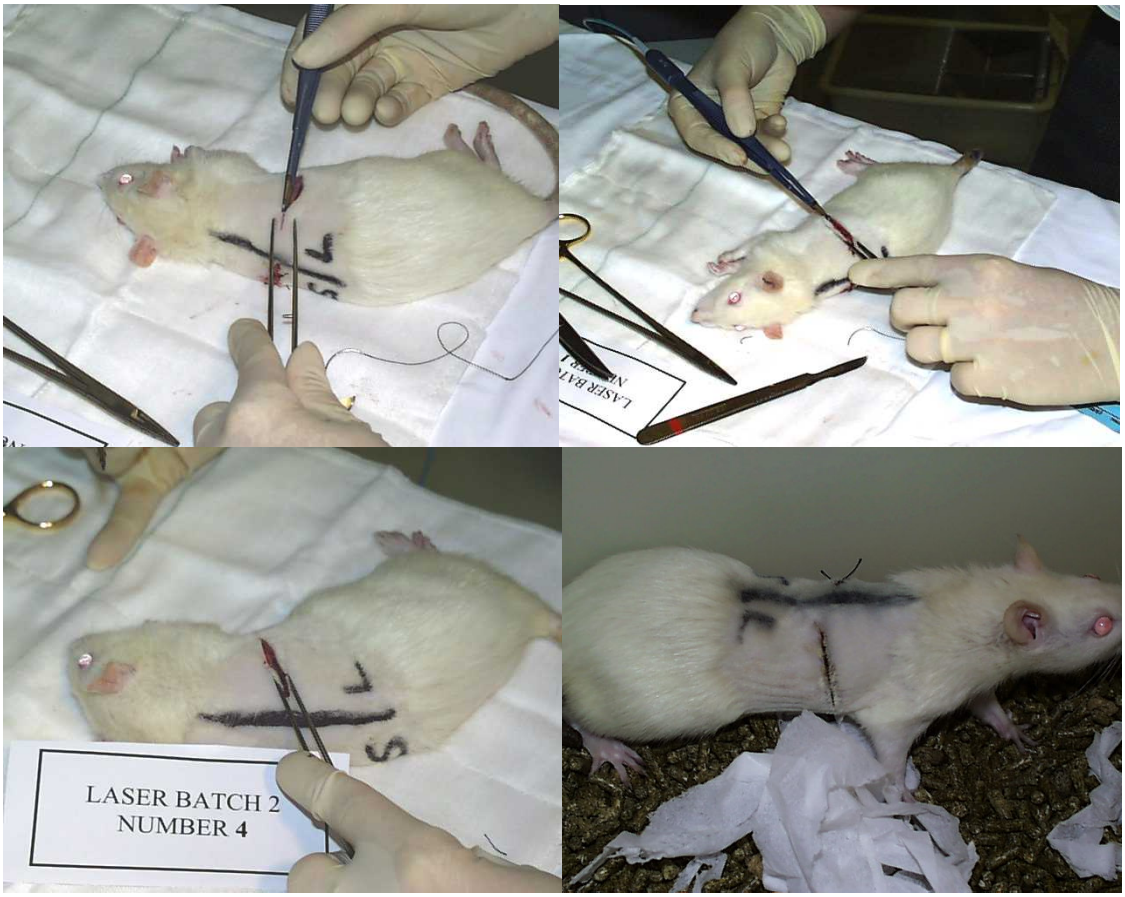


Figure 5-13 Welding process – Clockwise from top left (a) welding dermis with bipolar probes (b) welding top layers of skin (c) thermal relaxation of skin (d) wound at 1 day

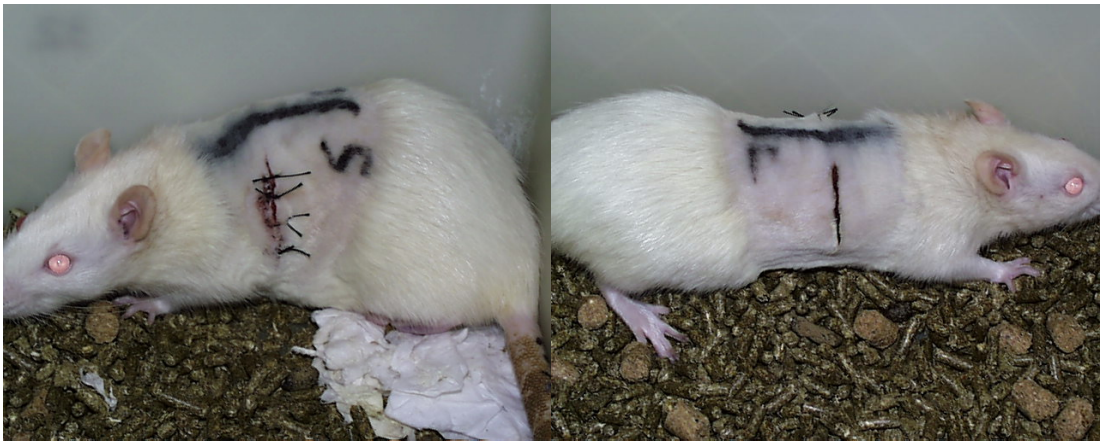


Figure 5-14 Suture and laser wound at 1 day

Extra care was taken when punching the test samples to avoid damage from undue force at the wound site. The necessity for care was shown when in one instance a sample from an early healing time ruptured along the wound by the downwards force of the punching.

The samples were punched as shown schematically in Figure 5-15, and care was taken to ensure that the wound was midway in the punch template. This approach ensured that the strength reading referred to the wound itself and was not the result of stress concentrations near the jaws of the testing machine. See Figure 5-16 (a) and (b) for the wound location. Following the strength test the small sections of tissue from the test samples were mounted on a card (dermis side down) and placed in a phosphate buffered formalin (10%) solution for subsequent histological analysis.

Each sample was supported while being mounted in the test clamps to ensure the weight of the sample and the attached sand paper did not pre-stress the wound. This was particularly necessary during the early healing phases when the resultant break force was extremely low for both lasered and sutured specimens. The resultant breakforce versus elongation data were plotted for each test specimen.

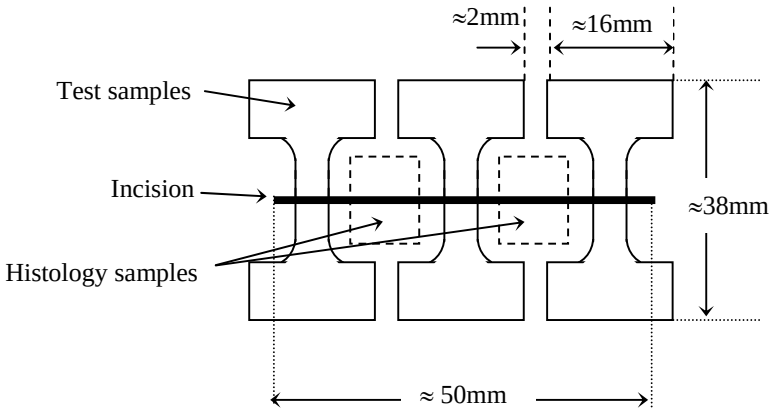


Figure 5-15 Test samples taken from wound area

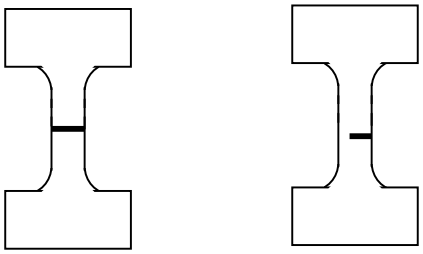


Figure 5-16 Wound position on test specimen (a) correct position (b) incorrect placement

Figure 5-17 Lasered test sample on sand paper prior to mounting in clamps

6 Analysis and Discussion of Results

The results of the experimental program are presented in two sections in this chapter. The first section presents the results for uninjured skin in terms of yield force and elongation of the test specimen at rupture. The literature search revealed little comparative data in this area and the few results that were found were difficult to replicate. The collected data were used to compare the healing of lasered and sutured skin. Results of the comparison are presented in the second half of this chapter.

6.1 Tensile Testing

The variation in the skin strength of rats was investigated to provide a base line for subsequent analysis. Base line strengths were needed to check for any significant variation in strength over the lengths of the applied wounds and for comparison with healed strengths at the end of the experiment. In total some 450 uninjured skin samples over the 21 sample positions from 19 rats were tested to investigate macro differences in strength by location and gender. Limitations in the supply of rats limited the results found by gender and age. However it was evident that both these parameters along with position influence the tensile strength of the skin at the different positions.

Only five rats were available specifically for this phase of the project. However a supply of extra rats became available after the experimental program commenced and these were used to increase sample sizes. These extra rats were not controlled for age or gender. Only five (5) of the total nineteen (19) rats were male. The 450 uninjured skin samples were collected over the 21 sample positions to provide estimates of baseline strength and elongation. The number of samples collected and tested from positions 11, 14, 18, and 21 was less than other positions for female rats. This was due either to the small size of the rat skin or to thick layers of fat and connective

tissue which did not allow samples to be reliably and accurately punched and tested. Male rats were on average larger and only 2 positions (11 and 14) had significantly smaller sample numbers. Table 6-1 and Table 6-2 show the final number of test samples actually tested from each position for female and male rats respectively.

Table 6-1 Number of samples tested from each test position- Female rats

Sample positions 1 to 21 Number of samples taken from each position																			
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
19	19	19	15	19	19	16	19	19	18	11	19	18	11	19	19	19	11	19	19	11

Table 6-2 Number of samples tested from each test position- Male rats

Sample positions 1 to 21 Number of samples taken from each position																			
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
5	5	5	5	5	5	5	5	5	5	2	5	5	2	5	5	5	4	5	5	5

All recorded yield forces for both male and female rats were associated within the narrowest region of the test specimen as shown in Figure 6-1. In contrast to the results published by Haut (1989) and Mendoza and Milch (1964) baseline test specimens did not consistently fail at the centre of the test specimen. Revision of the notes taken on the testing revealed that only specimens from positions 1, 2, 5, 8, 9, 12 and 15, routinely broke in the middle. Other specimens broke either towards the bottom or top of the specimens, as shown in Figure 6-2. This supports the existing evidence of Langer's Lines or the animal equivalent whereby the stress-strain relationship is closely associated with the microstructural organisation of collagen.

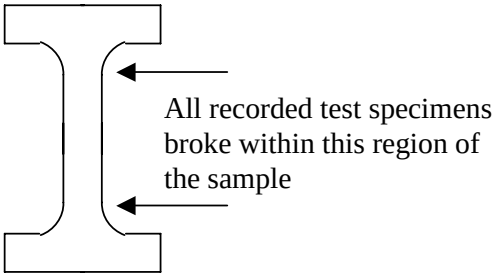


Figure 6-1 Limits of break site on test specimens

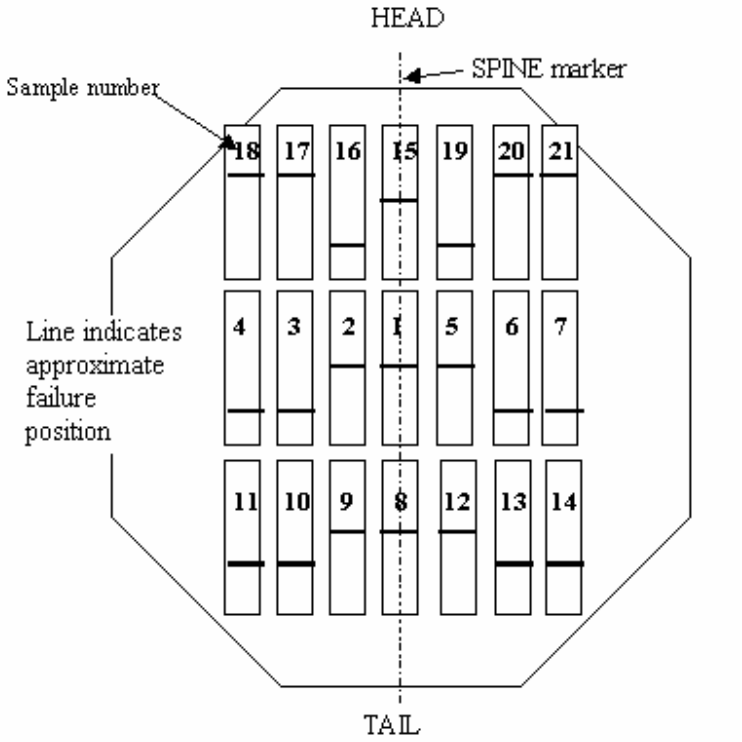


Figure 6-2 Approximate locations of specimen failures

Typical tensile responses for various test positions are shown in Figure 6-3 to Figure 6-7. The work by Haut (1989) presents the yield *stress* in pascals (Mpa) whilst Mendoza and Milch (1964) uses p.s.i. (pounds per square inch). No corresponding data is given for the cross sectional area of the sample, measurement technique nor variations in these measurements. Haut states that the thickness of the samples was measured using an "electrical contact sensing micrometer probe" however it does not indicate if the hair, subcutaneous fat or connective tissue was removed either before these measurements were taken or before the tensile testing. While the fat and connective

tissue would make no significant difference to the yield force they would make considerable difference to the measured cross sectional area and hence to the calculated break force.

In this project it was chosen to report the strength results in terms of yield force (in Newtons) instead of yield stress or force per cross sectional area. The tensile strength of the specimen was recorded as the maximum force in Newtons applied at failure and the elongation in millimetres of the specimen was measured from the first non zero point to the main failure point as shown in Figure 6-3.

The failure characteristics of the specimens could be a function of the preferential orientation of collagen fibres or possibly differences in the degree of cross-linking of the fibres for one direction. In approximately 50% of the tests the failure of the specimen was complete as indicated by the test profile in Figure 6-3 and Figure 6-5. They return to zero applied force with no further extension. In the remaining 50% of specimens the failure of the connective tissue and fat layers is evident as shown in Figure 6-4 and Figure 6-6 for males and females respectively. These secondary failures tended to be, but were not limited to, positions 10, 11, 13, 14, 4, 7, 17, 18, 20 and 21. Figure 6-7 indicates the profile of a specimen that slipped in the grips in the testing phase. Figure 6-8 shows a sample mounted in the grips and testing machine prior to testing.

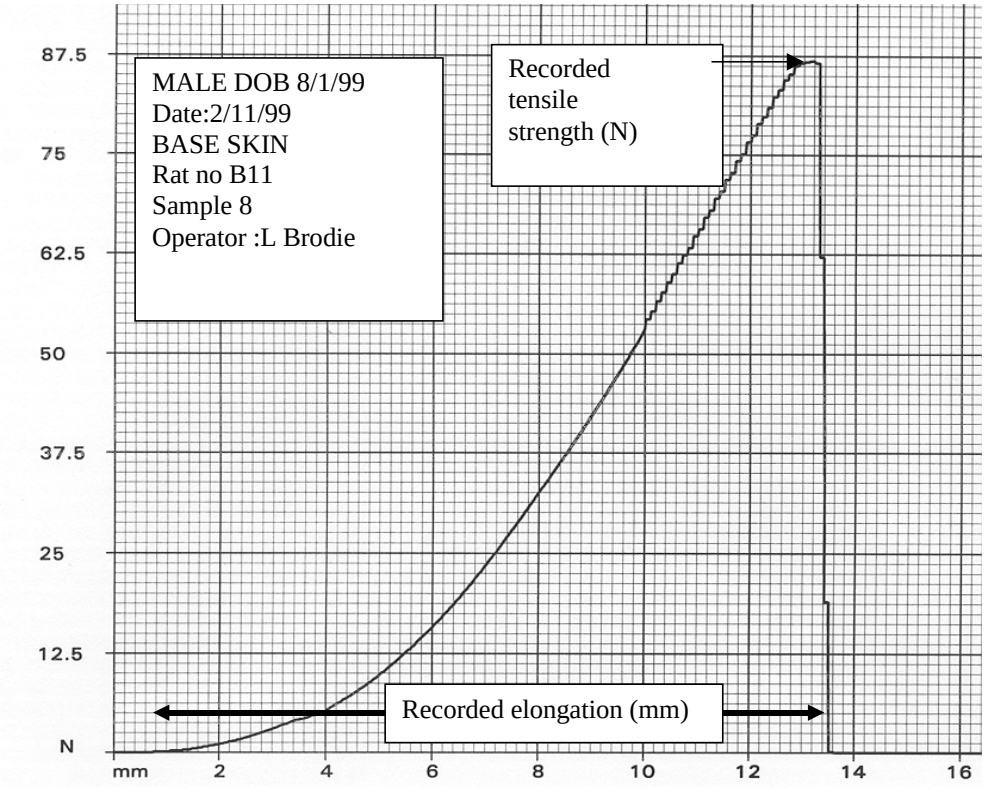


Figure 6-3 Typical tensile test response of complete skin failure - Male

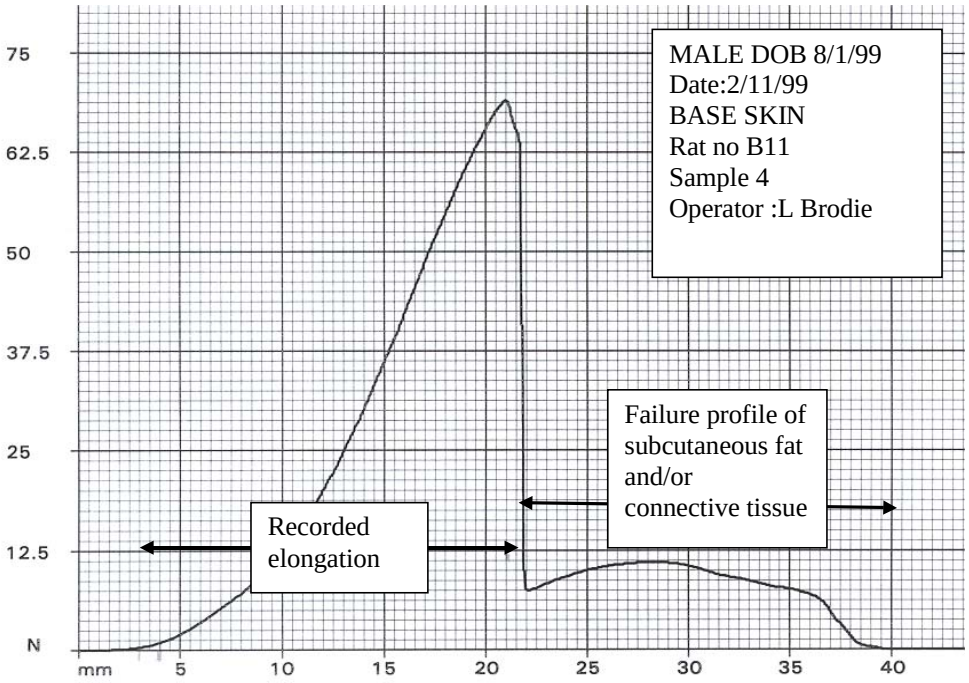


Figure 6-4 Typical tensile test response of connective tissue – male

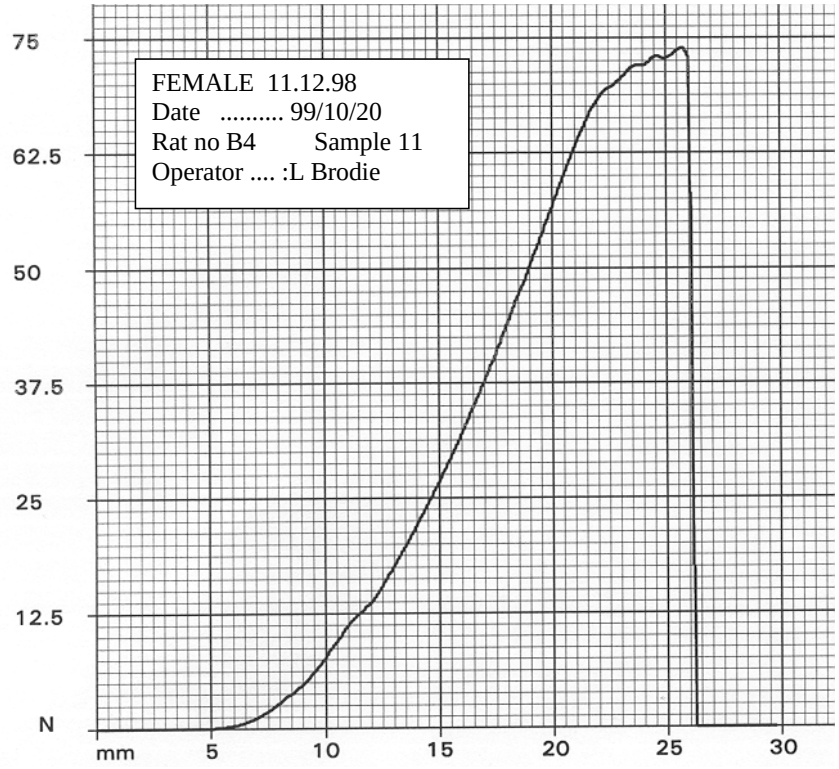


Figure 6-5 Typical tensile test response for complete skin failure – Female

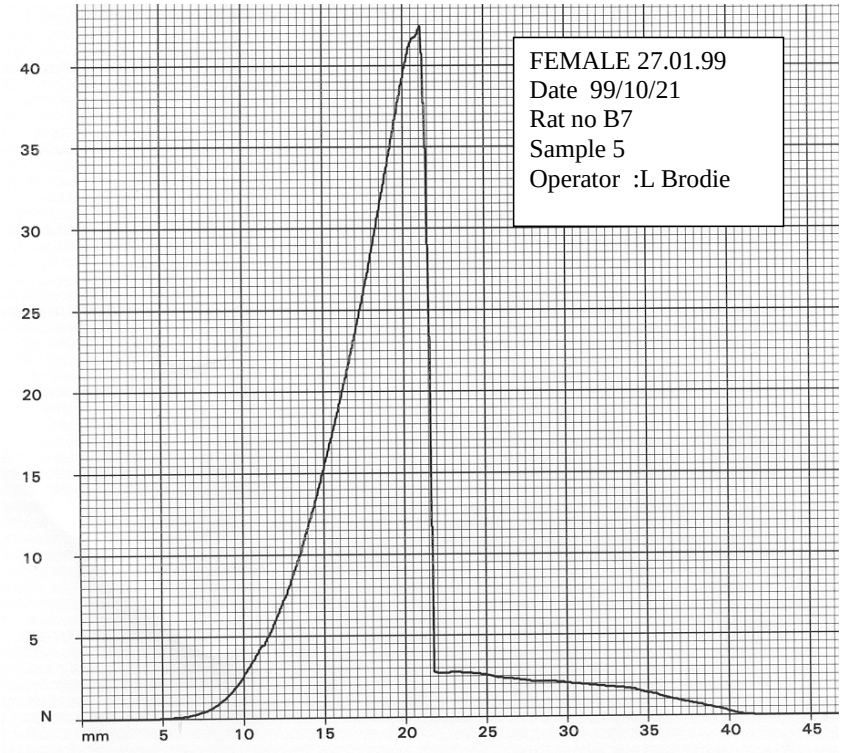


Figure 6-6 Typical tensile test response of connective tissue - Female

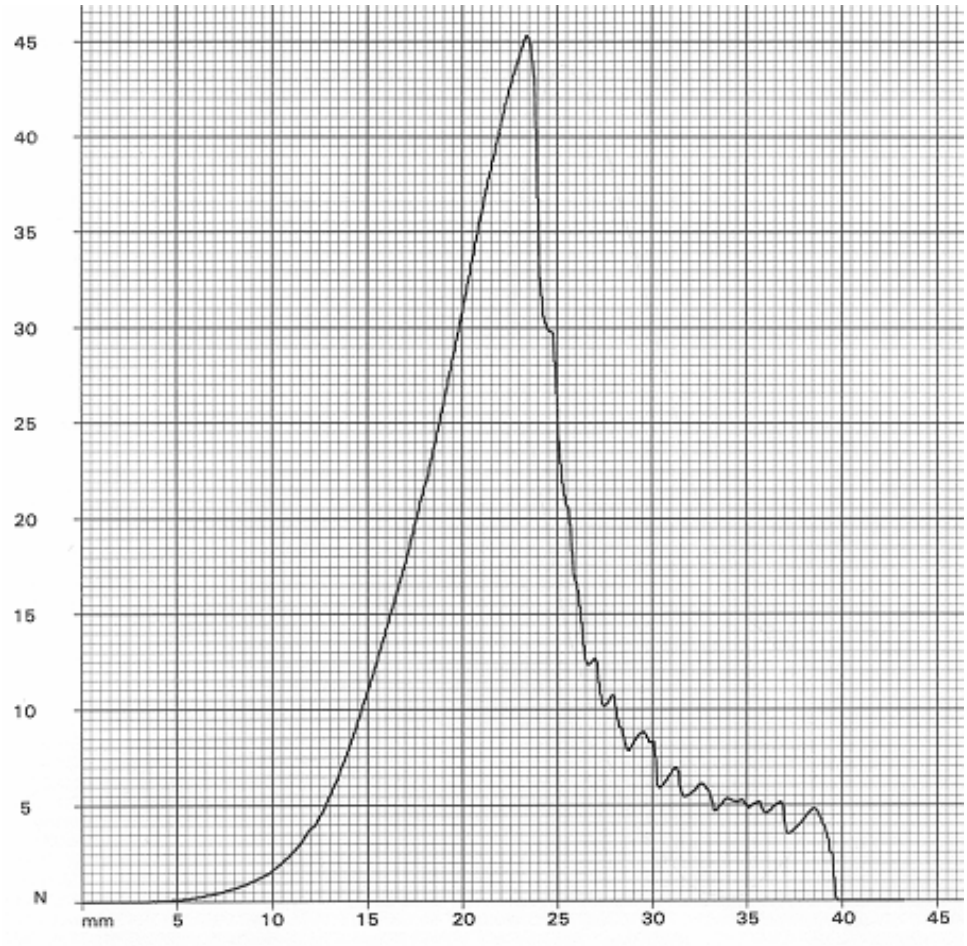


Figure 6-7 Typical tensile test response indicating slippage

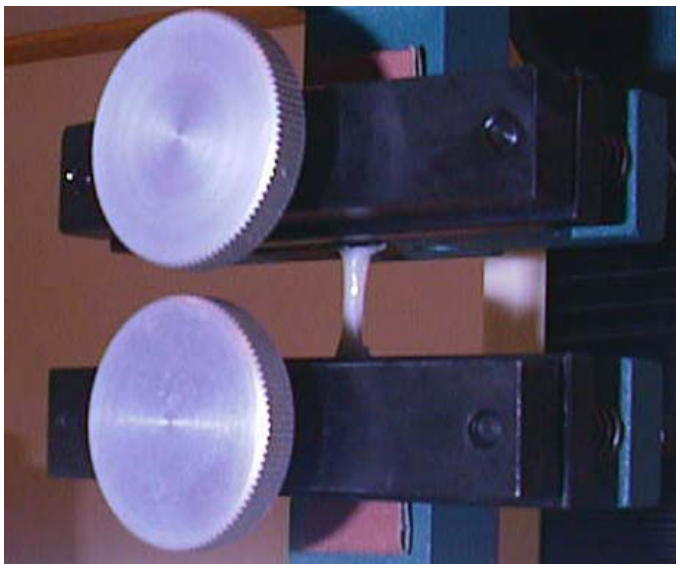


Figure 6-8 Test specimen in clamps of testing machine

6.1.1 Yield Force – Female

Figure 6-9 plots measured skin strength data from the 21 positions for female rats. Outlying data points were subsequently investigated by reference to the laboratory notes. To do this the average and standard deviation of all points for each position was calculated and marked on the graph. Any points outside these margins were examined for possible errors. A data point was declared to be an outlier when it occurred more than one standard deviation from the mean at each location. One data point (position 17, break strength 8.27 N) was eliminated due to documented specimen slippage during testing.

The available literature suggests that age is a significant factor in the tensile strength and elongation of the skin. No such relationship was obtained in this investigation because the initial test methodology allowed for rats to be matched in age and sex. Only one rat was significantly younger than the others and while its test results did support age as a relevant factor the statistically significant relationship could not be obtained. Figure 6-10 shows yield force plotted against age for selected positions for female rats shows how most of the data were obtained from 270 to 300 day old rats. The measured data from the young rat was eliminated as a confounding factor in subsequent analysis. All other points were included in the recalculating of the average and standard deviations as no reasonable reason could be found to exclude them as true outlying points.

The normalised standard deviations were calculated as a proportion of the mean tensile strength for each rat and are plotted in Figure 6-11. This graph shows the standard deviations for each of the main lines of testing - head, midline and tail. The deviations are symmetrical about the spine indicating the variation in tensile strength is due to normal variations in individual rats and is not

an experimental error. All remaining data points were concluded to be within the natural limits of the properties of skin.

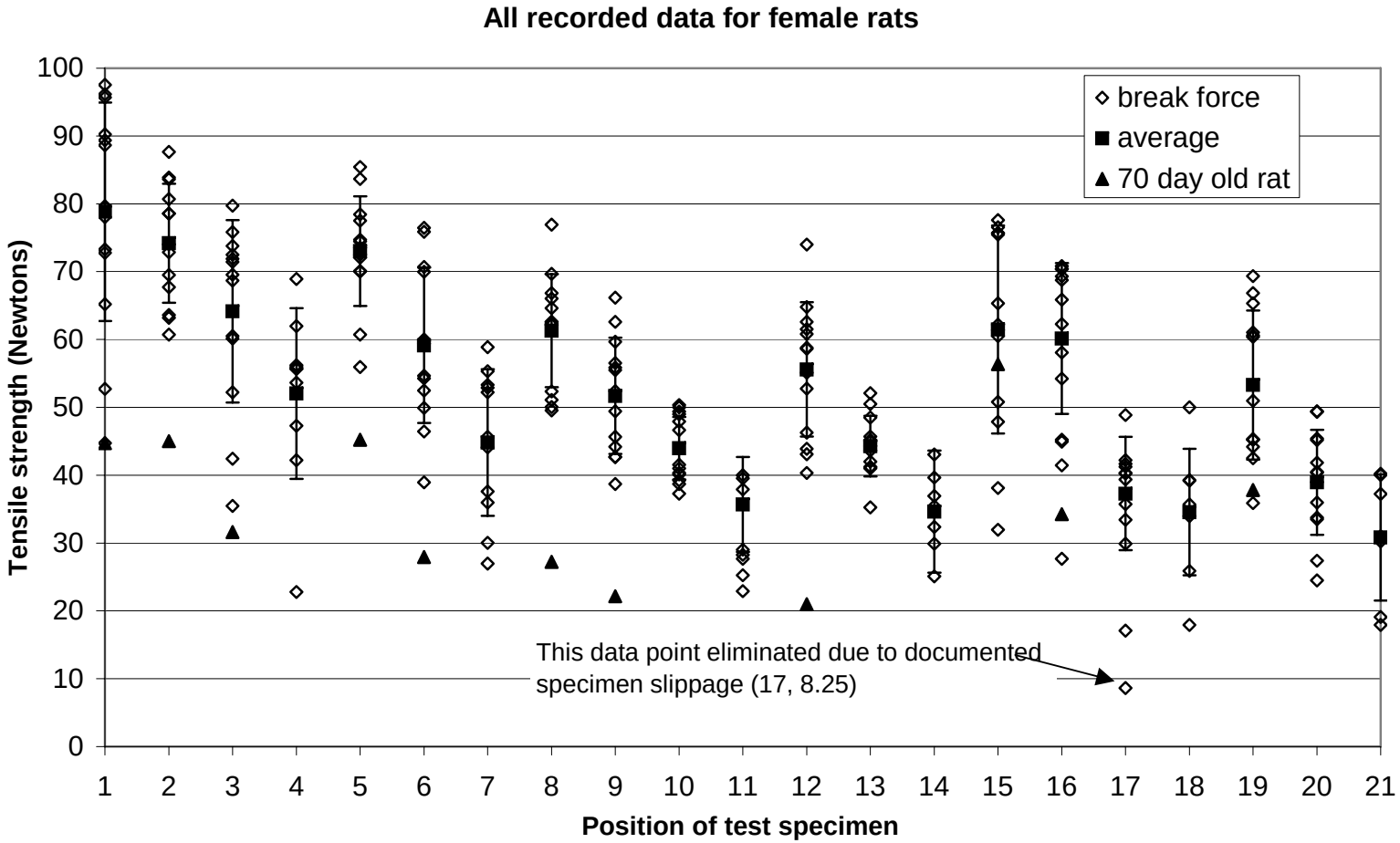


Figure 6-9 Female mean tensile strength

Figure 6-10 Significance of age and position on tensile strength

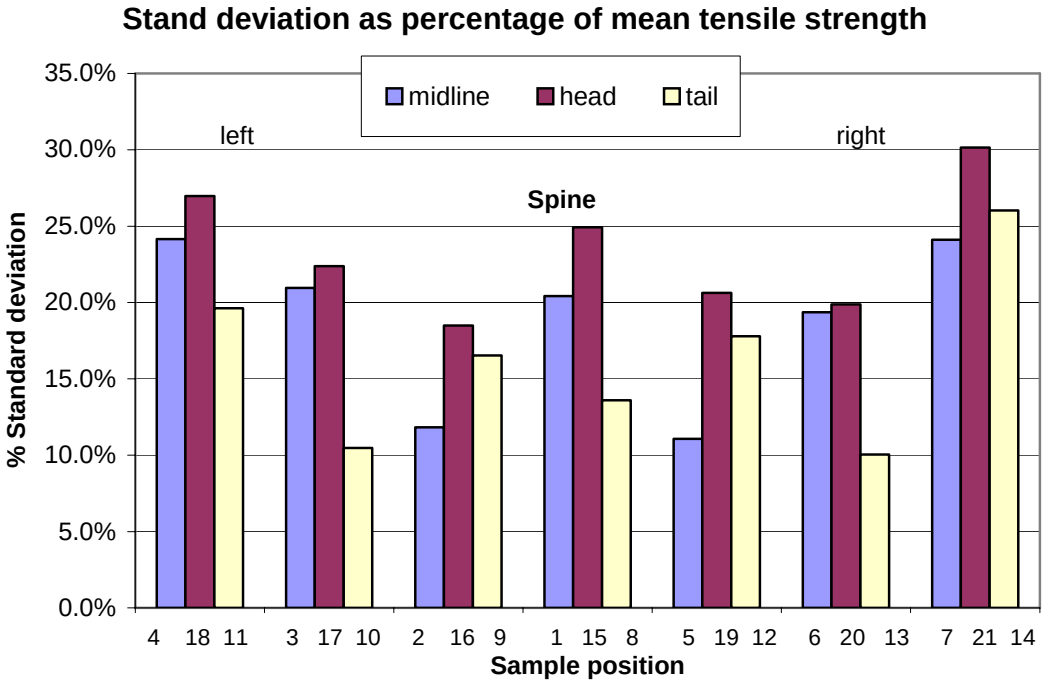


Figure 6-11 Normalised standard deviations for related positions for female rats

Graphs of all data points, means and standard deviations for female rats are shown in Figure 6-12, Figure 6-13 and Figure 6-14 respectively for the head, midline and tail positions of female rats. Differences in strength from the spine outwards towards the head and the tail are statistically significant. The data have large standard deviations reflecting both natural differences found in most biological data and also experimental error. Larger sample sizes, which might have reduced the variation, were not ethically possible in this project.

Experimental factors inevitably contributed to larger than expected standard deviations shown on these graphs. After rats were sacrificed the spine was marked on the skin by laying the rat in a prone position and identifying the position of the spine by touch. The layers of fat and loose connective tissue allowed the skin to move relative to the spine easily between 1-1 ½ centimetres, with the result that the marking and positioning of subsequent punch specimens contained this degree of potential error. No methodology was available to remove this error.

Previous work in by Haut (1989) indicated relatively smaller deviations though with smaller sample sizes. Haut indicated standard deviations of 8.3%, 9.7%, 2.8%, 14.2%, 15.4%, 5.6% of the mean tensile stress for each of the six age groups between 1.5 and 6 months. Due to the small sample sizes (n=3) these figures for standard deviation are of limited value. Reports by Mendoza and Milch (1964) with sample sizes of 24 reported standard deviations of 37.0%, 27.4%, 26.8%, and 37.7% of mean tensile strength for 'old male', ‘old female', 'young male' and 'young female' respectively. These values are much closer to those found in this project.

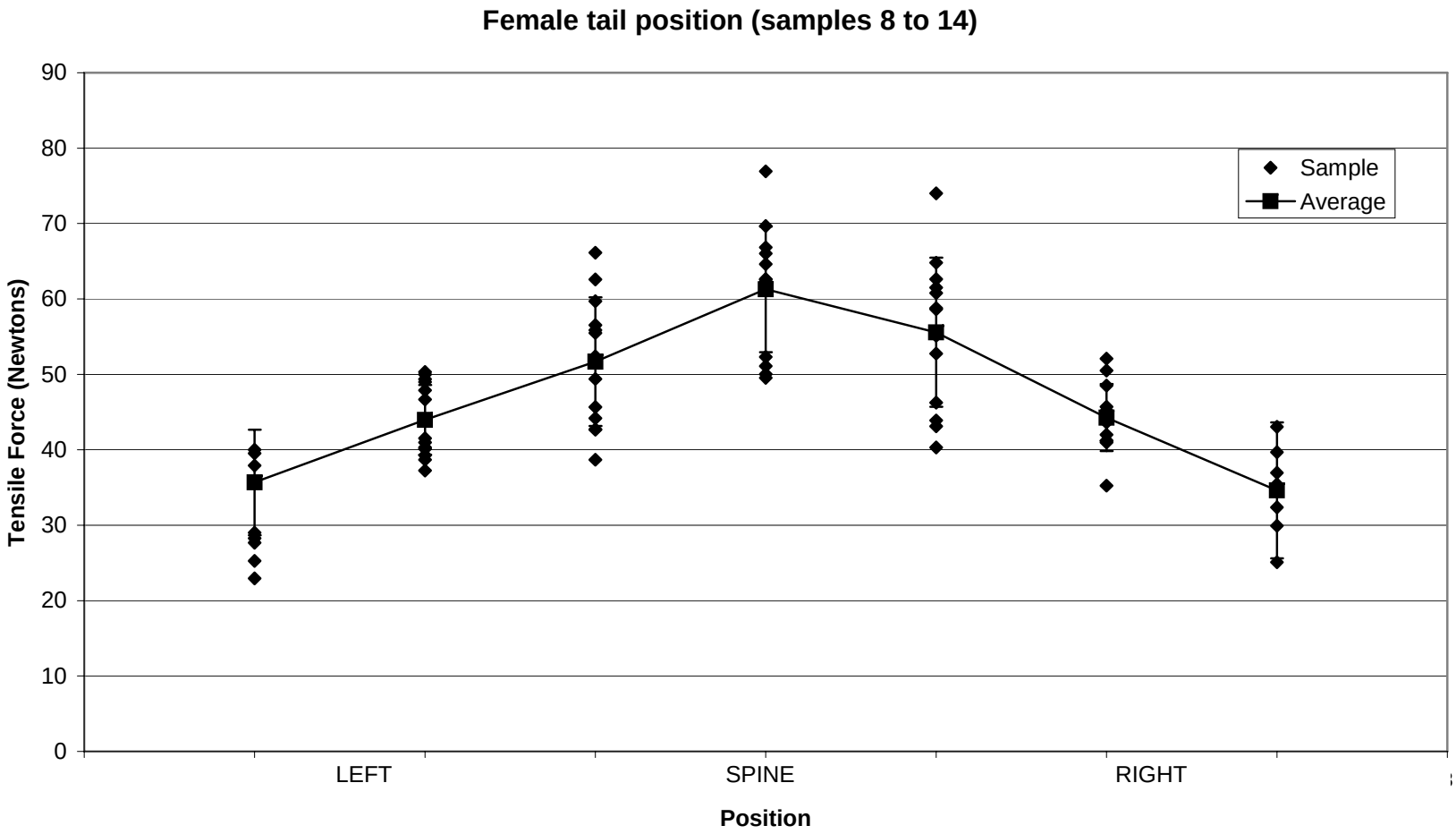


Figure 6-12 Female tail break force data

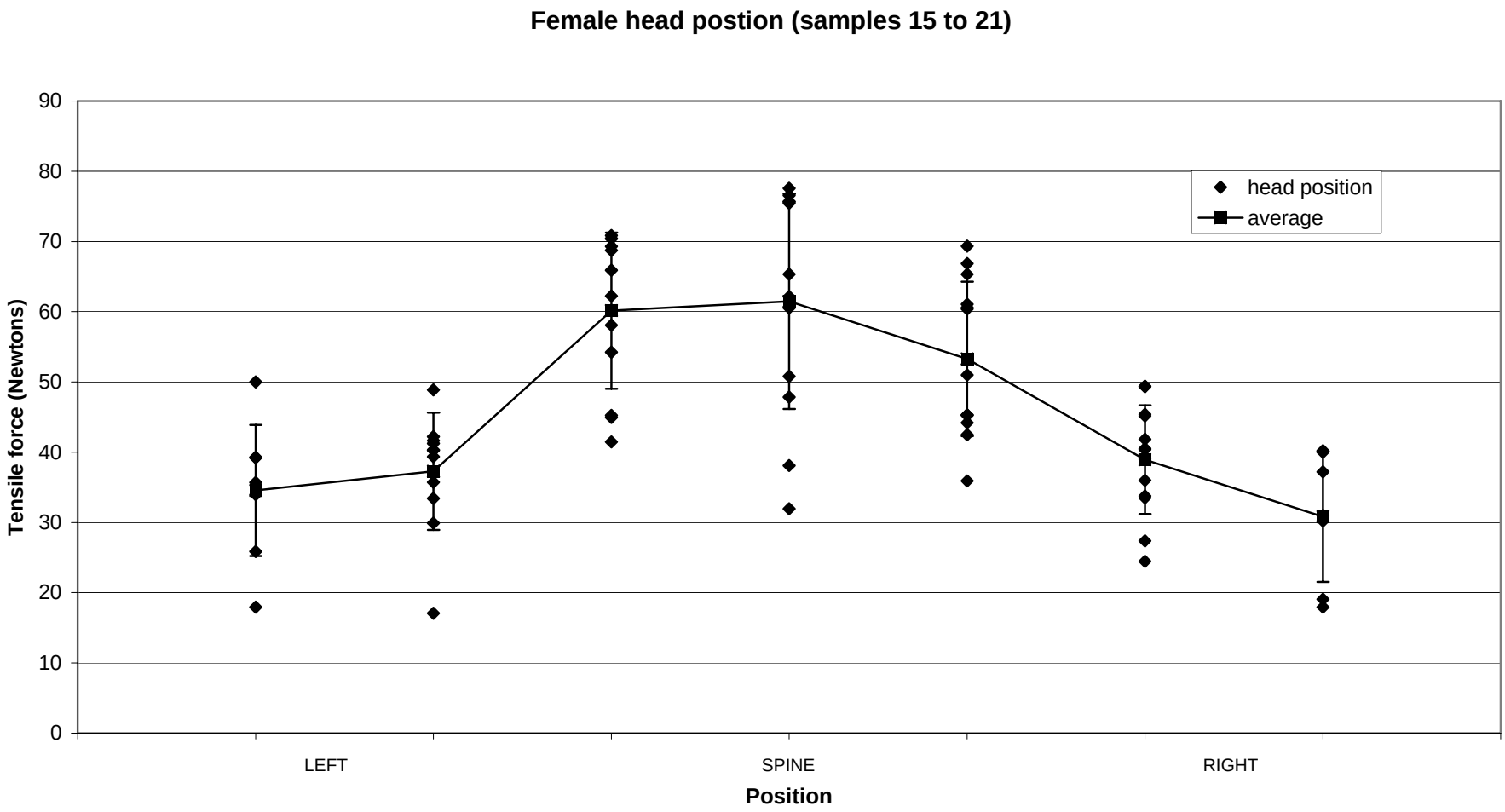


Figure 6-13 Female head break force data

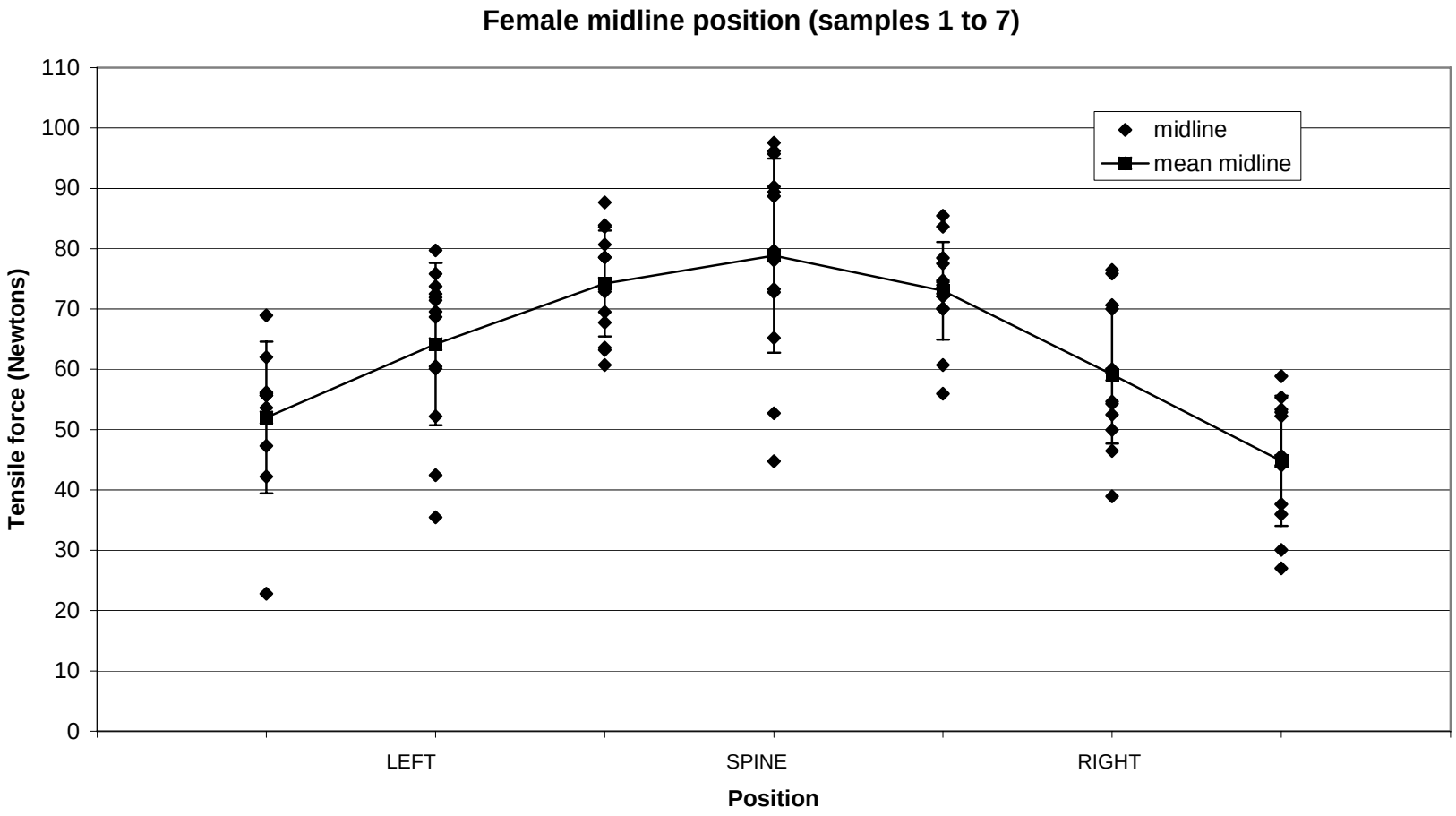


Figure 6-14 Female midline break force data

6.1.2 Elongation – Female

The data for the elongation of test specimens for female rats is presented in Figure 6-17, Figure 6-18 and Figure 6-19 for the head, midline and tail positions respectively. No comparative published data for the elongation or elasticity of rat skin was found. As with the yield force there were large stand-deviations due to natural variations in the rats. The standard deviations ranged from approximately 5% to 16% of the mean elongation depending on the position. The largest deviations were recorded from positions 3 and 6. These two positions are symmetrical about the spine in the midline position. Normalised standard deviations for elongation were calculated and plotted in Figure 6-16. All deviations are symmetrical about the spine indicating that the recorded deviations are due to normal variations in rats and not experimental error.

Literature suggests that the elongation is influenced by load, loading rate, direction of sample (parallel and perpendicular to the spine) age and maturation (Vogel, 1981). Investigation of the elongation of rat skin was not an aim of this project and measurements were taken at a single set strain rate to remove this influence. Samples were matched for age, sex, loading rate, and orientation of sample with respect to the spine.

The results do show that the elasticity of the skin is greatest near the spine and most variable in this region. The skin closer to the head is different to that lower in the body, being more elastic near the forelegs.

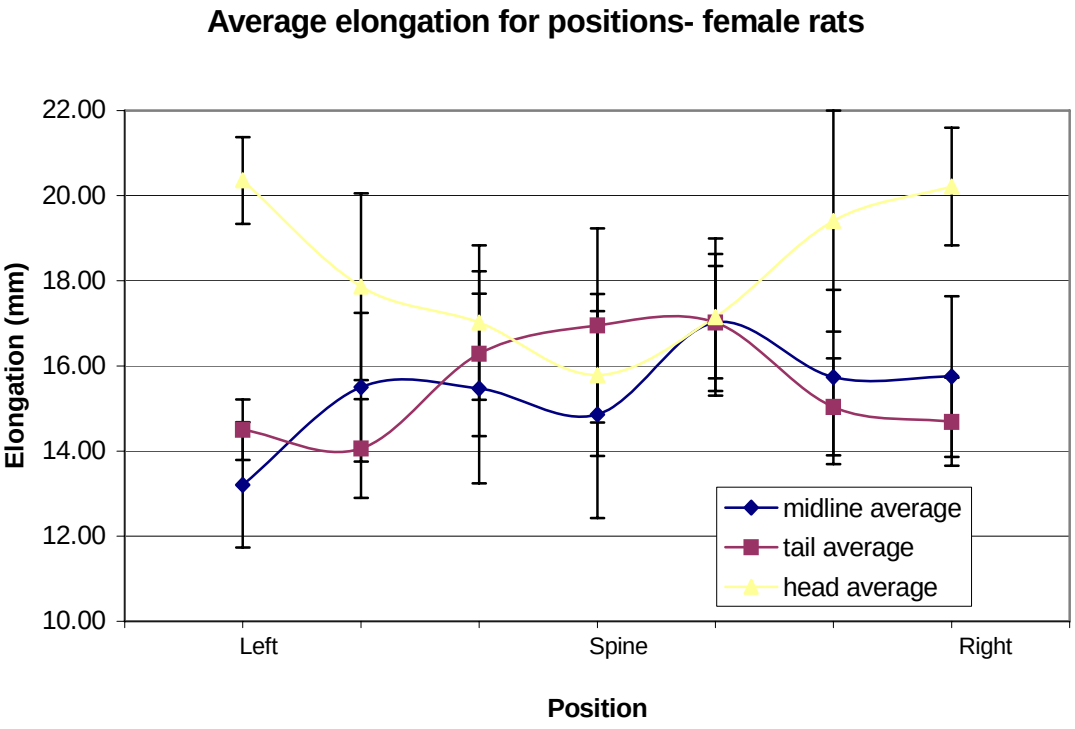


Figure 6-15 Average elongation for each position for female rats

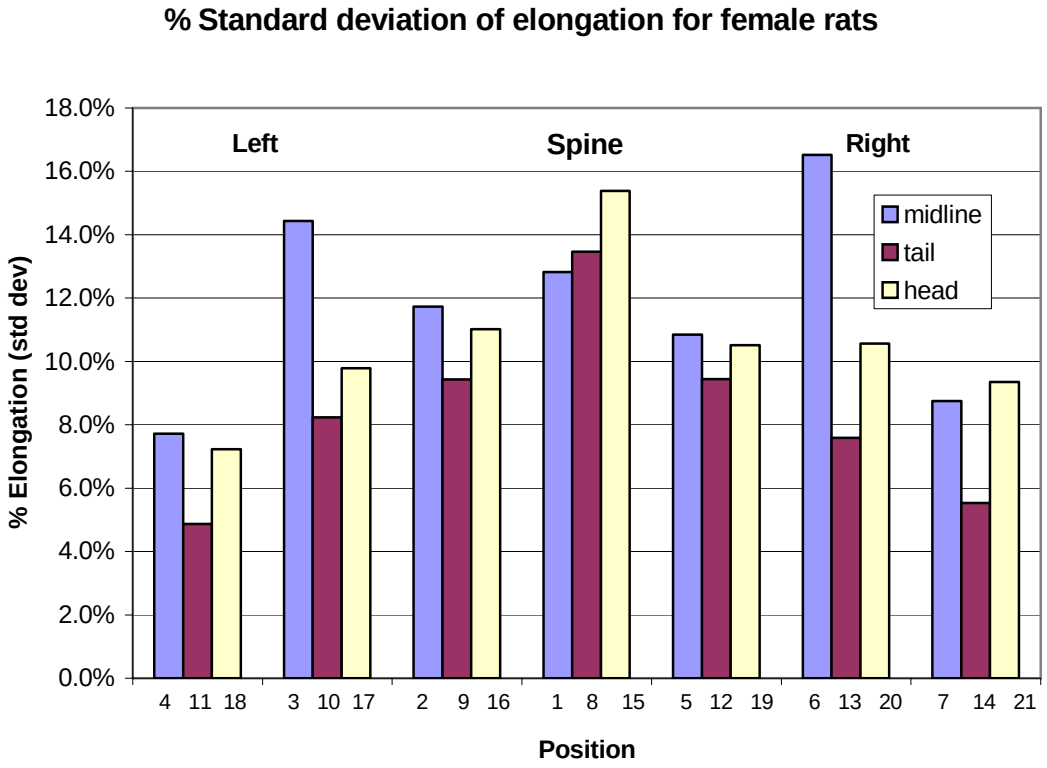


Figure 6-16 Normalised standard deviations of elongation for each position

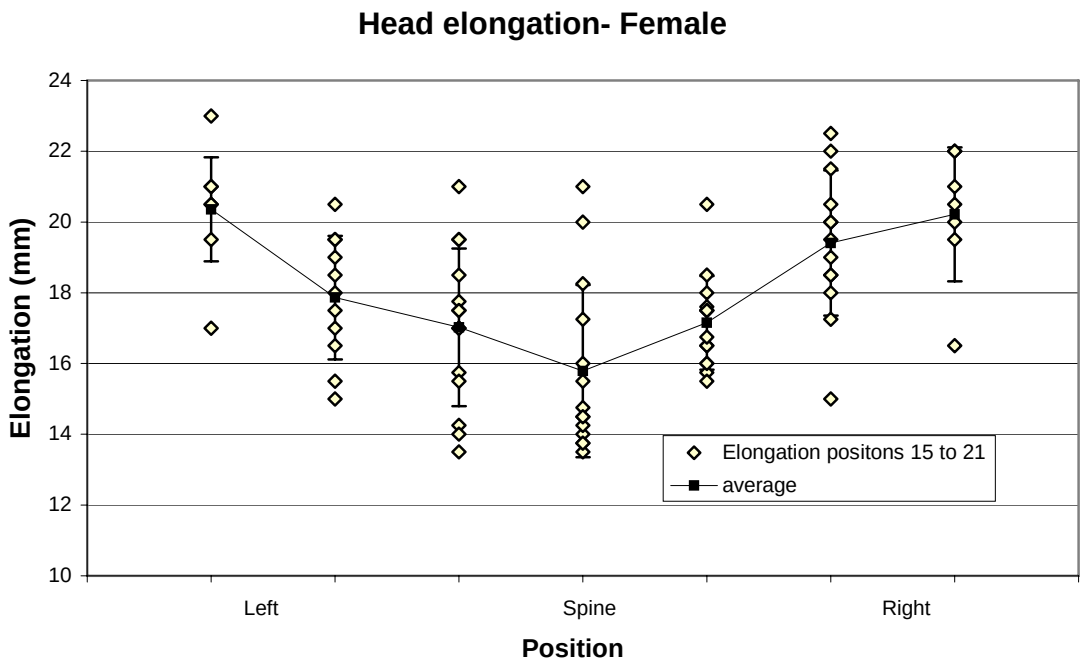


Figure 6-17 Elongation for head line - Female

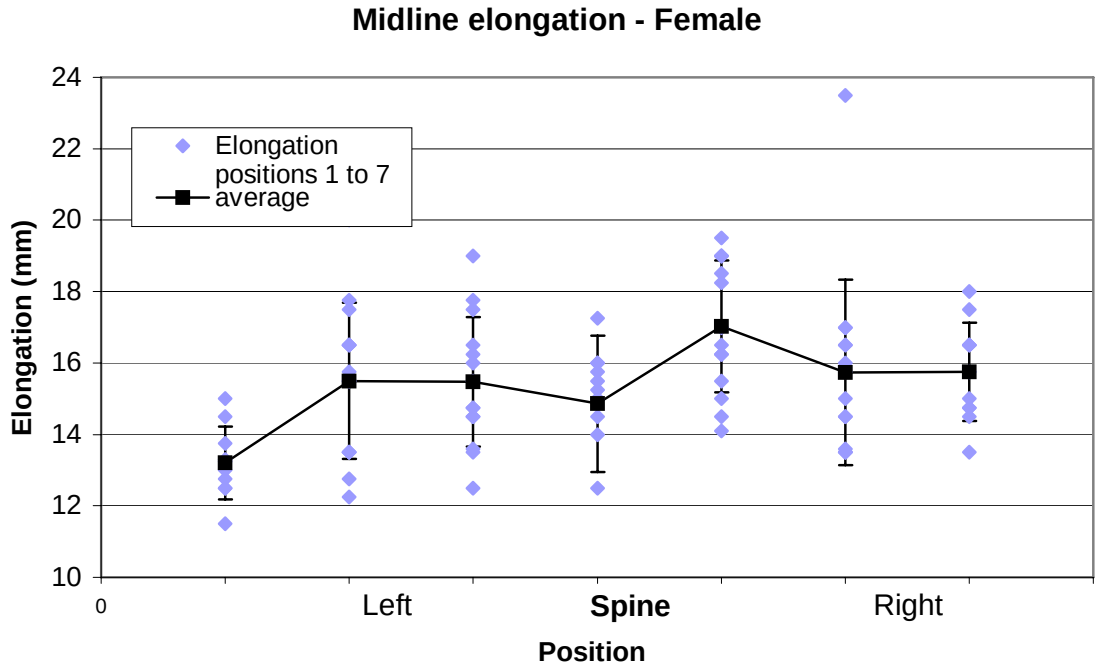


Figure 6-18 Elongation for midline - Female

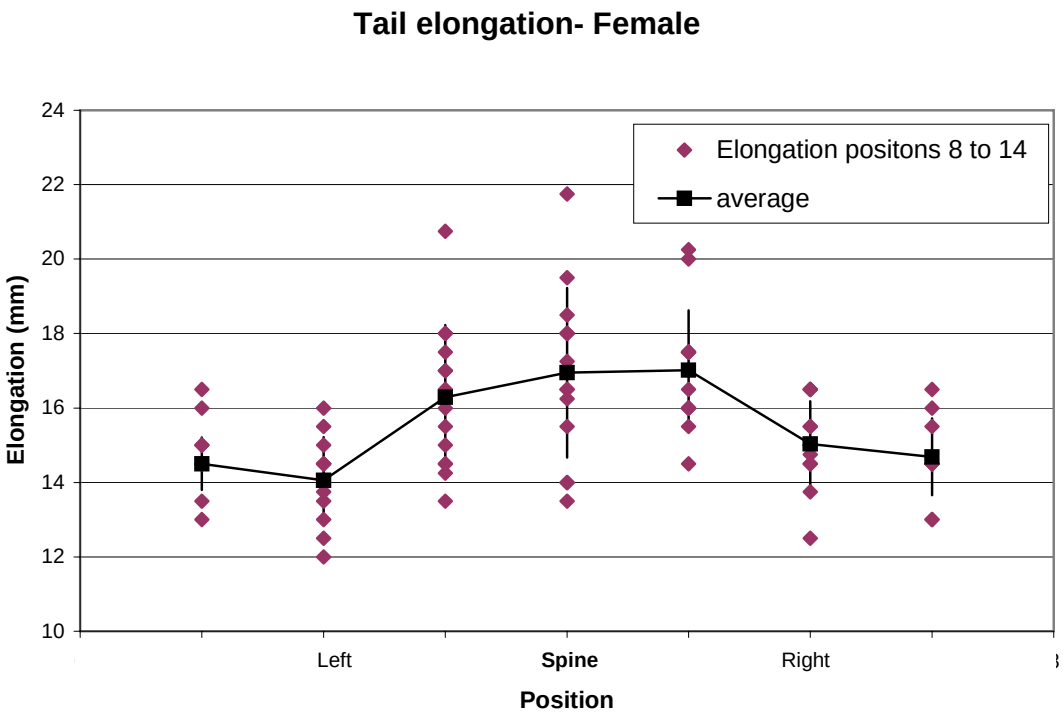


Figure 6-19 Elongation for tail line - Female

6.1.3 Yield force - Male

Figure 6-20 shows a plot of all measured skin strength data in each of the 21 positions for male rats. This plot includes all outlying data points. As with the data for female rats the mean and standard deviation of all points for each position was calculated and plotted on the graph. Any points outside these margins were examined for possible errors. A data point was declared to be an outlier when it occurred more than one standard deviation from the mean at each location.

Figure 6-21 analyses data by looking at individual rats. Rat number 3 (B13) is outside one standard deviation lower than the mean in a majority of sample positions – 4, 7, 9, 12, 13, 17 and

20. It has no recorded data for position 11 and 14 and notes taken during testing indicated it was smaller physically. It was however matched in weight rat. All male rats were of similar ages (298 to 301 days). There was insufficient evidence to provide a reason for excluding the consistently low tensile strength values from the analysis.

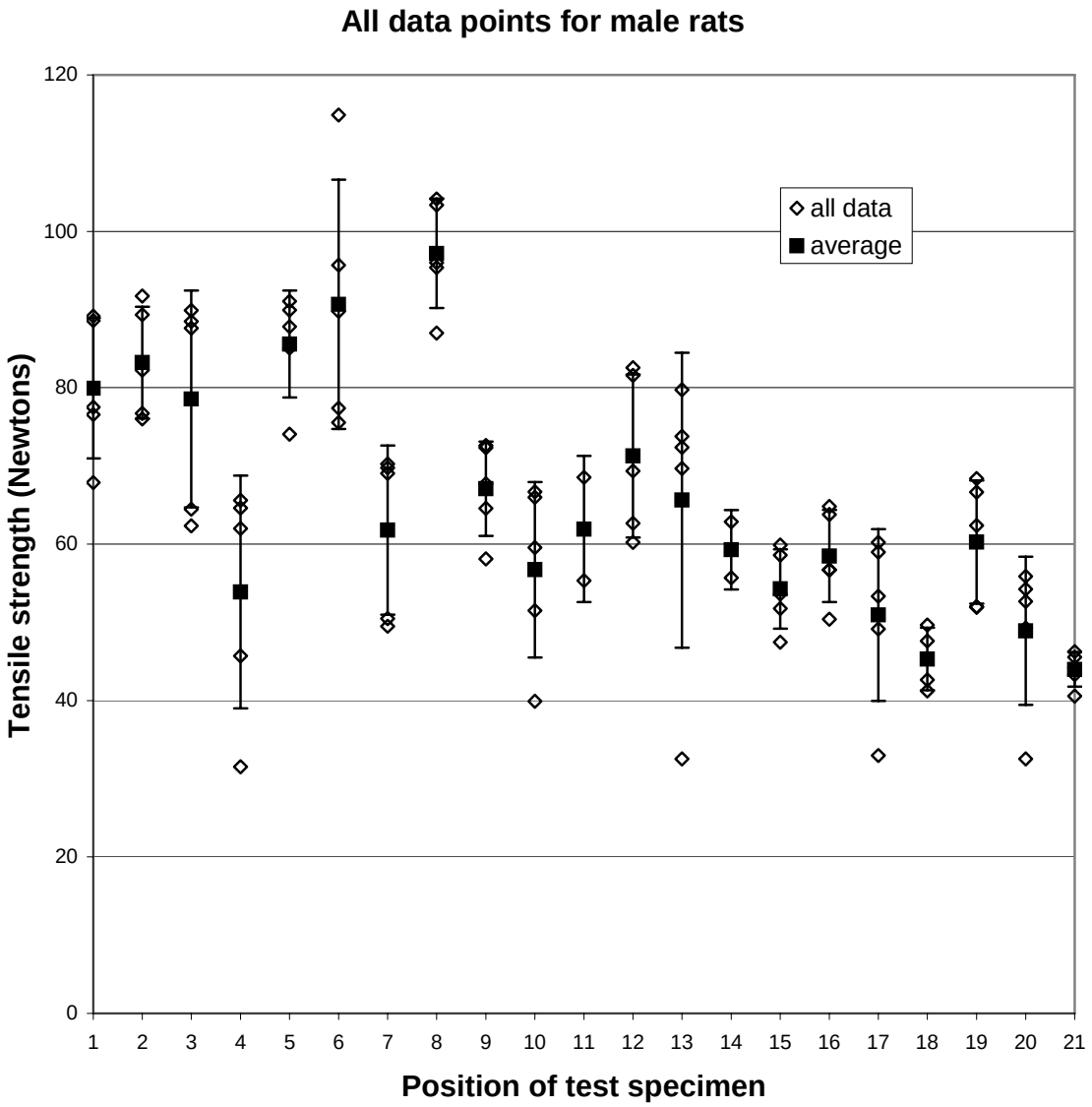


Figure 6-20 Male mean tensile strength

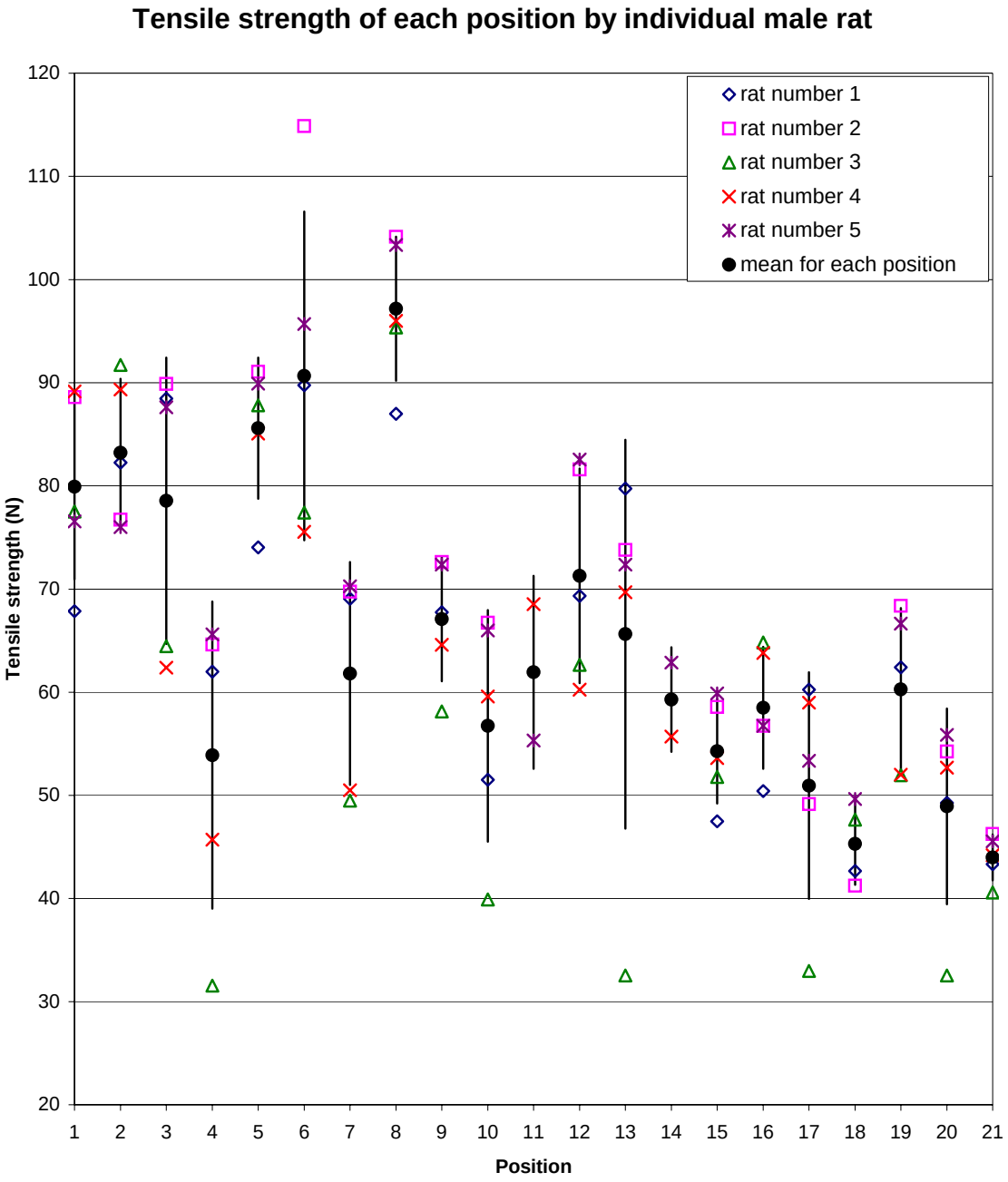


Figure 6-21 Tensile strength data from individual male rats

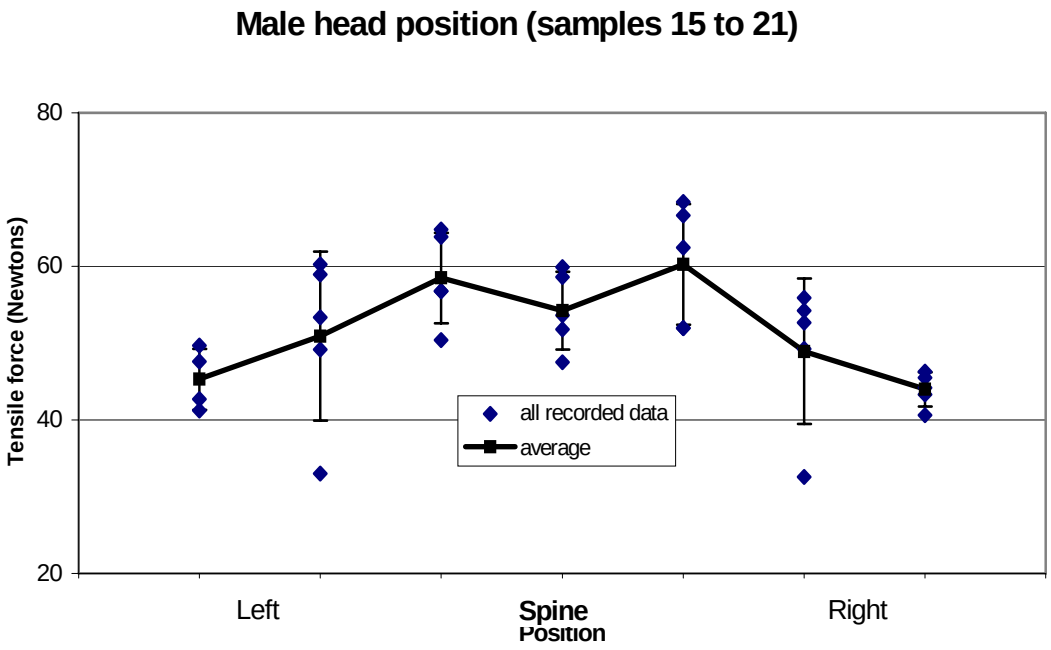


Figure 6-22 Male head break force data

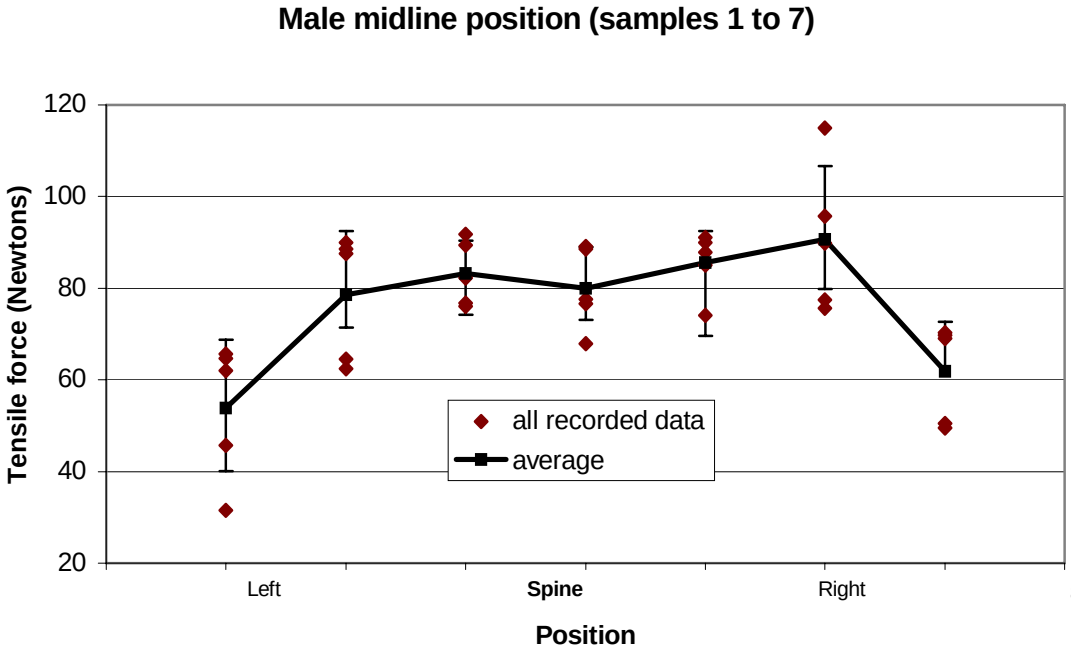


Figure 6-23 Male midline break force data

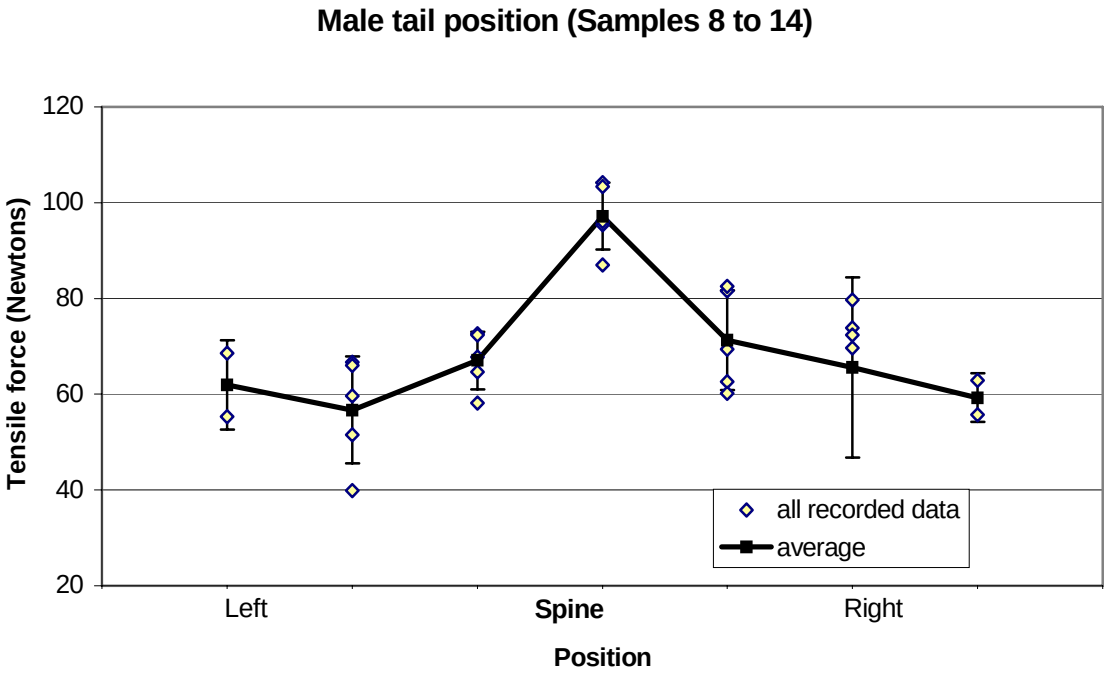


Figure 6-24 Male tail break force data

Figure 6-25 and Figure 6-26 shows the mean strength results by location on female and male rats respectively. There were insufficient data to establish any significant difference between males and females although trends indicate that this may be the case. Mendozer and Milch (1964) and Fry et al (1964) claim to have found differences in skin strength between male and female rats with the male rats having higher average values.

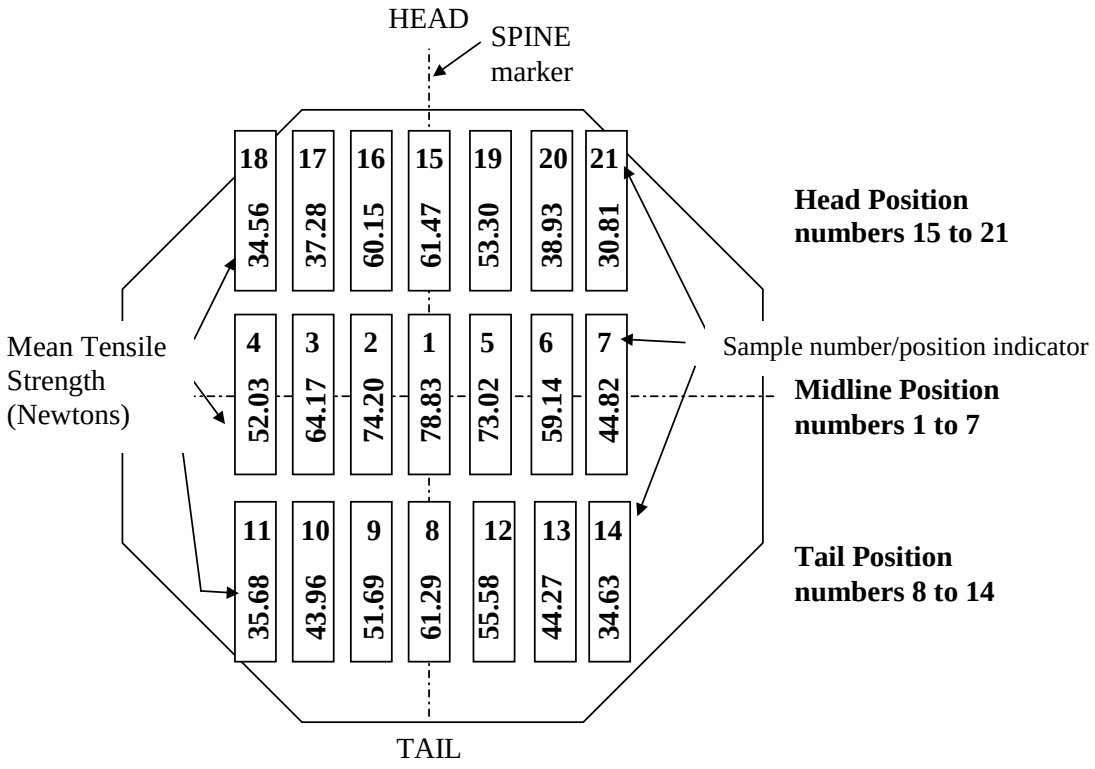


Figure 6-25 Female mean tensile strength by position

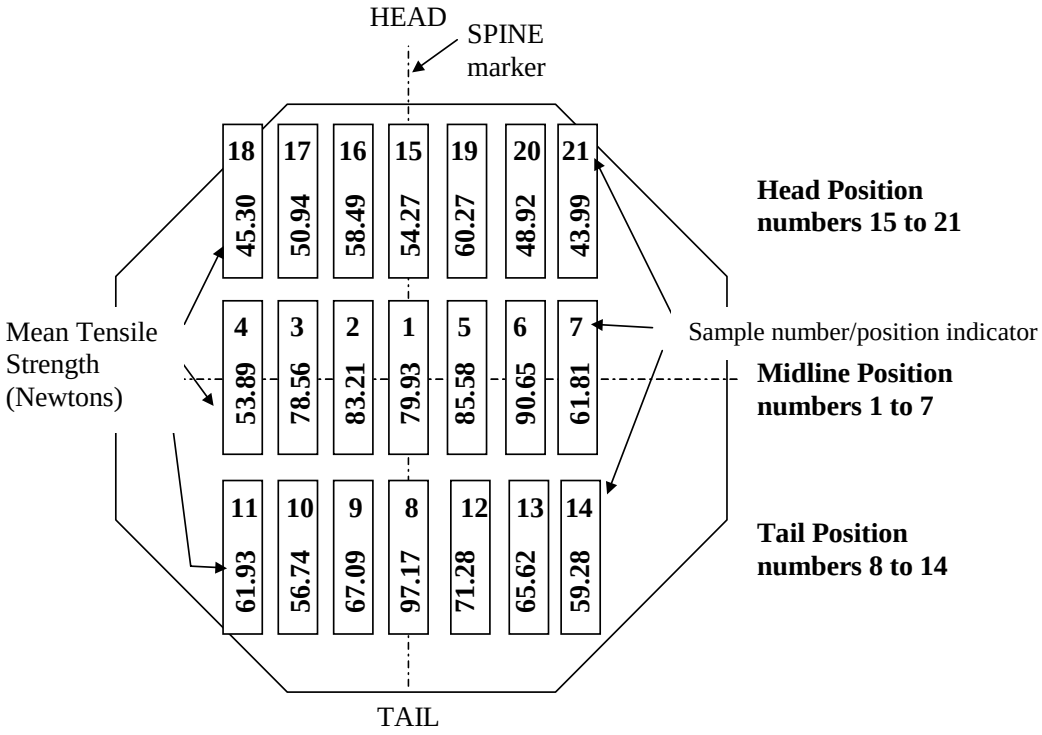


Figure 6-26 Male mean tensile strength by position

6.2 Statistical model for baseline strength

Regression analysis was carried out on the data. A model was established using the predictors of position of sample, sex and age and tested at the P = 0.05 level. While all three predictors have an effect the overall regression only explains 39% of the variation in the data as shown in Table 6-3.

Table 6-3 Model summary for baseline strength

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.484(a)	.234	.230	16.77393
2	.583(b)	.340	.333	15.61399
3	.634(c)	.402	.392	14.90674

a Predictors: (Constant), position of sample
b Predictors: (Constant), position of sample, sex
c Predictors: (Constant), position of sample, sex, age days

The small sample size for male rats limited the analysis and results for this population. Analysis of the female population for uninjured skin indicated that position was significant but age of the rat was not. (P = 0.05)

The ANOVA results from this regression are summarized in Table 6-4. The table shows that the regression is highly significant and the predictor of position explains 66% of the variation in the measured strength values.

Table 6-4 ANOVA table of yield strength

Regression Statistics	
Multiple R	0.82832156
R Square	0.6861166
Adjusted R Square	0.66389477
Standard Error	9.7562218
Observations	243

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	16	47022.0277	2938.87673	30.8757873	5.9752E-48
Residual	226	21511.5532	95.1838638		
Total	242	68533.5809			

6.3 Laser Power Settings

The laser setting, the corresponding temperature in the skin and the weld effectiveness was evaluated. The laser was set to a variety of power settings and the skin temperature recorded. A table of resultant temperatures and observations is given in Table 6-5. The temperature readings were mainly obtained from the calibrated bead thermistors as the infrared thermometer proved difficult to focus accurately on the target spot and hold steady long enough for an accurate reading. The manipulation of the forceps and bipolar laser probes for the welding technique (described in section 5.5.2) often interfered with the infrared beam adding to the inaccuracy of the reading.

The rats were sacrificed immediately following this procedure and the welds closely examined. Viable welds were achieved with power settings from 8 to 20 watts in both modes of operation. With the high power settings carbonisation was visible and there was considerable smoke production. This came mainly from the hair.

The recorded temperatures in the pulsed mode are higher than for the CW mode. This is contradictory to published data although the published data does not consider the application of

contact probes. No published data was found using contact probes. Sanders and Reinisch (2000) published that pulse durations and frequency can be used effectively to limit thermal damage to surrounding tissue. This indicates a lower temperature. This work was done with a CO_2 laser in free beam mode. The absorption characteristics of the Nd:YAG laser is significantly different and the effect of the contact probes has not been previously investigated. The pulse duration and frequency and the thermal characteristics of the contact probes may produce a significantly different effect.

After analysis of the weld (visual analysis only), the recorded skin temperatures and smoke production a power setting of 10 watts, CW mode was chosen for the welding phase of this investigation. One rat was anaesthetised and the wound welded at these parameters. The rat was then allowed to recover to ensure that the welding was strong enough for the rat to move and healing to take place. Refer to Figure 6-27. The wound remained closed until fully healed and did not appear to cause undue discomfort to the animal.

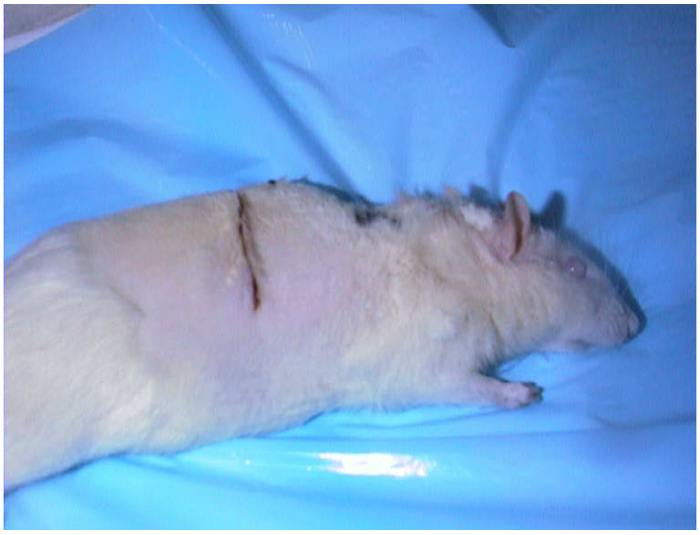


Figure 6-27 Completed wound closure with laser

Table 6-5 Comparison of laser settings and skin temperature

Laser Power Setting (watts)	**Mode**	**Temperature (^{O}C) ± 1 ^{O}C**	**Observations**
5	CW	40	Difficulty in welding wound in a reasonable length of laser application
5	Pulsed	42	
8	CW	45	
8	Pulsed	48	Weld achieved in approximately 5 seconds
10	CW	55	Viable weld achieved in approximately 3 seconds
10	Pulsed	60	Viable weld achieved, but significantly more smoke produced. This may have lead to cell necrosis due to overheating of the tissue in contact with the probe.
12	CW	62	
12	Pulsed	65	
15	CW	70	Viable weld achieved in less than 3 seconds but carbonisation of the tissue was visible in places and weld was difficult to control.
15	Pulsed	75	Weld was achieved but did not appear to be strong. Carbonisation was visible and occurred in short space of time. Hair on surface of skin caused considerable smoke.
20	CW	75-78	
20	Pulsed	85	

6.4 Laser vs Suturing Healing

Results from tests on twenty rats were used to compare the healing rates of sutured and laser welded wounds in phase 3. Rats were anaesthetised and incisions placed bilaterally perpendicular to the spine midway between the front and hind legs as described in Chapter 5. After predetermined periods of healing the rats were sacrificed, the skin excised and punched with the microtensile test die then tested to failure in the tensile testing machine.

Each rat provided a maximum of 6 test samples – 3 laser and 3 suture. Laser welded wounds were identified as number 2, 3 and 4 while suture wounds were 5,6 and 7 as on the positions of baseline tensile testing. This is shown schematically in Figure 6-29.

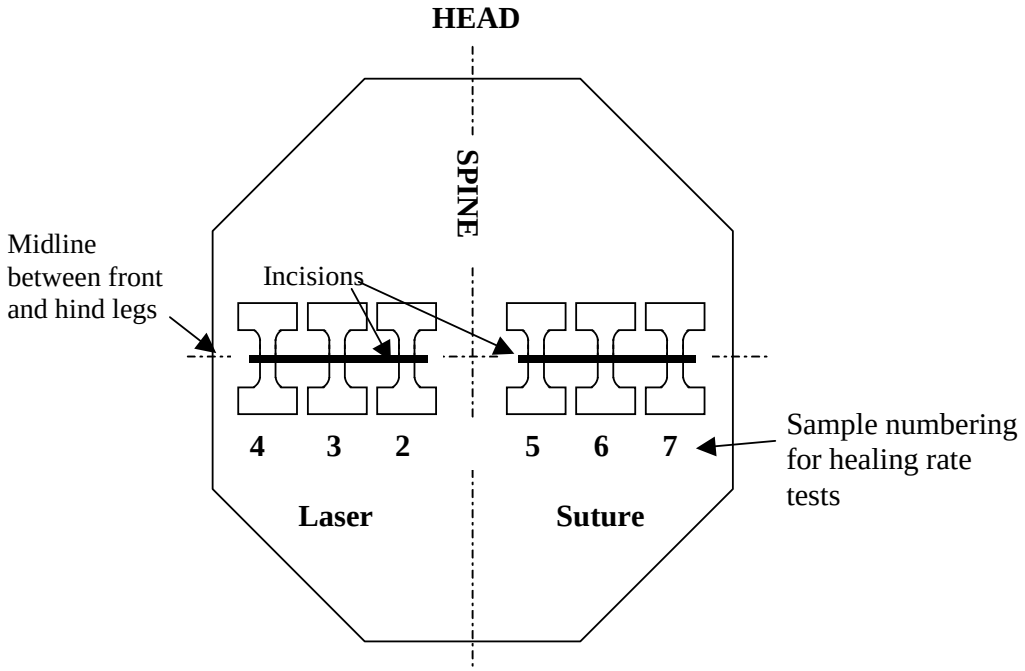


Figure 6-29 Sample numbering for healing rate tests

This testing program was designed to provide 120 samples but a number of samples were not suitable for testing. Total samples tested over a 91 day period totalled 54 laser and 51 sutured wounds. Causes for the reduction in sample numbers included one rat dieing inexplicably when injected with anaesthetic and in others stitches irritated causing rats to scratch and open wounds and rats removed and undid stitches. Figure 6-31 shows minor irritation to the skin by the stitches. Rat L1-2 gnawed stitches off a skin level so that they appeared to have been removed. On skinning the stitches were found to have remained subcutaneously causing irritation. The small holes in the skin where the stitches came through to the surface considerably weakened the wound and so these results were also eliminated from the analysis. Problems with wounds are detailed in Table 6-6.

From observation the laser welded wounds caused less discomfort to the rats than the stitches and the majority of wounds healed well. Figure 6-32 and Figure 6-34 shows the normal healing of a laser welded wound. Rats removed stitches themselves by scratching or grooming before day 7 when they were due for removal and no intervention was required. The removal of stitches by grooming and scratching is shown Figure 6-33 and normal healing of sutured wound is shown in Figure 6-35.

Table 6-6 Laser and Suture - notes of wounds

Rat identification number	**Number of samples provided**	**Notes**
L1-1	3 suture samples 0 laser samples	Rat had scratch laser wound. Wound had not reopened but insufficient healing had taken place for punching and testing.
L1-2	3 laser samples 0 suture	Stitches remaining subcutaneously causing irritation.
L2-1	3 laser samples 0 suture	Sutured wound had caused irritation and scratching had reopened stitches. Wound restitched twice. Rat continuously gnawed at wound causing severe irritation. Refer Figure 6-30. Rat was sacrificed at 7 days for humane reasons.
L3-2	3 laser samples 3 suture samples	Rat had scratched at stitches. Wound was given longer to heal (unattended) and rat was sacrificed as normal. Refer to Figure 6-31.
L3-5	0 samples	Died on being anaesthetised.

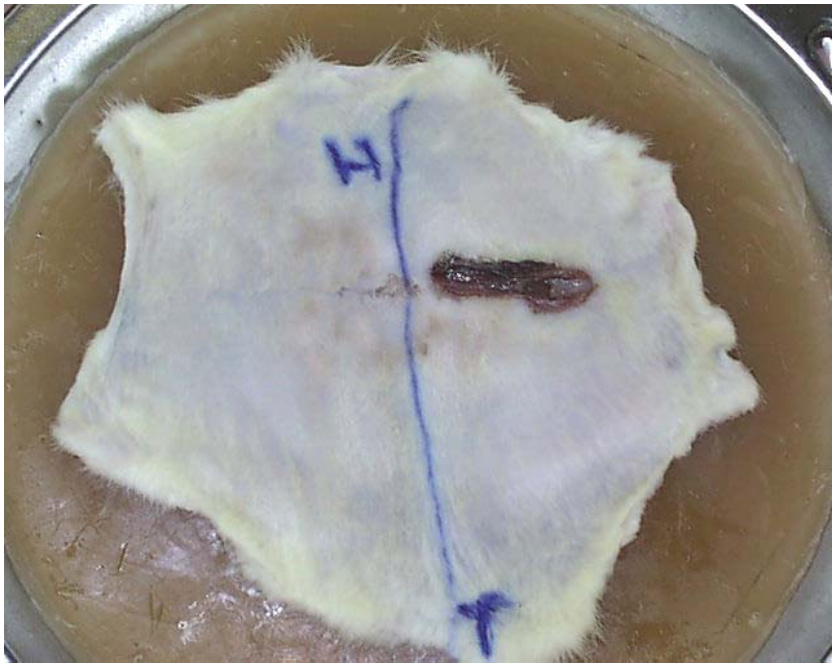


Figure 6-30 Severe irritation of wound

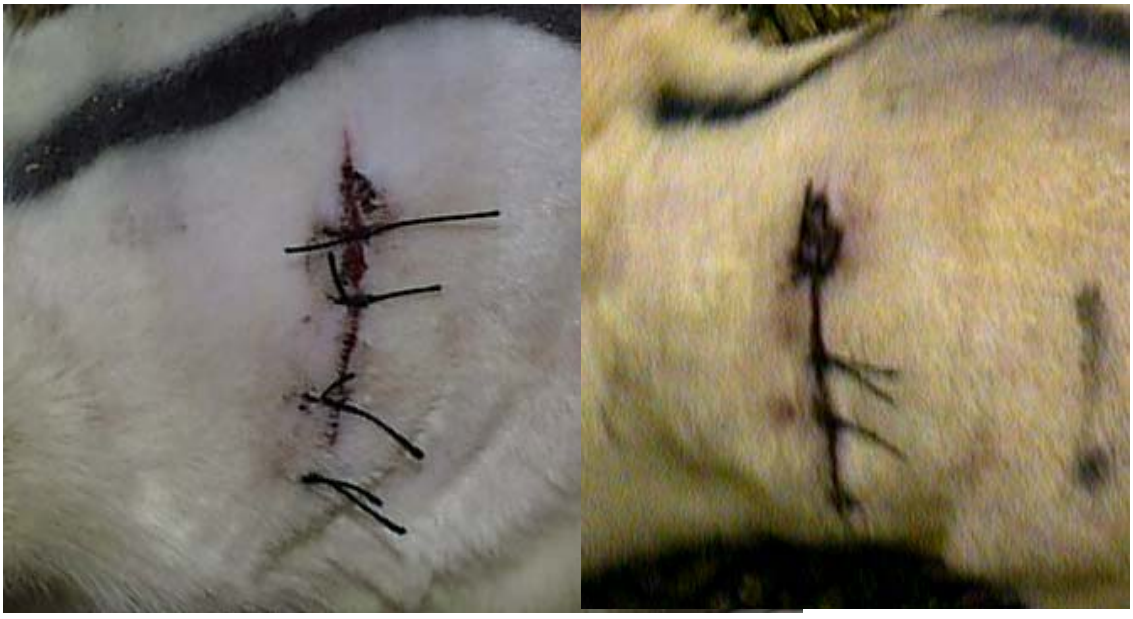


Figure 6-31 Irritation caused by stitches

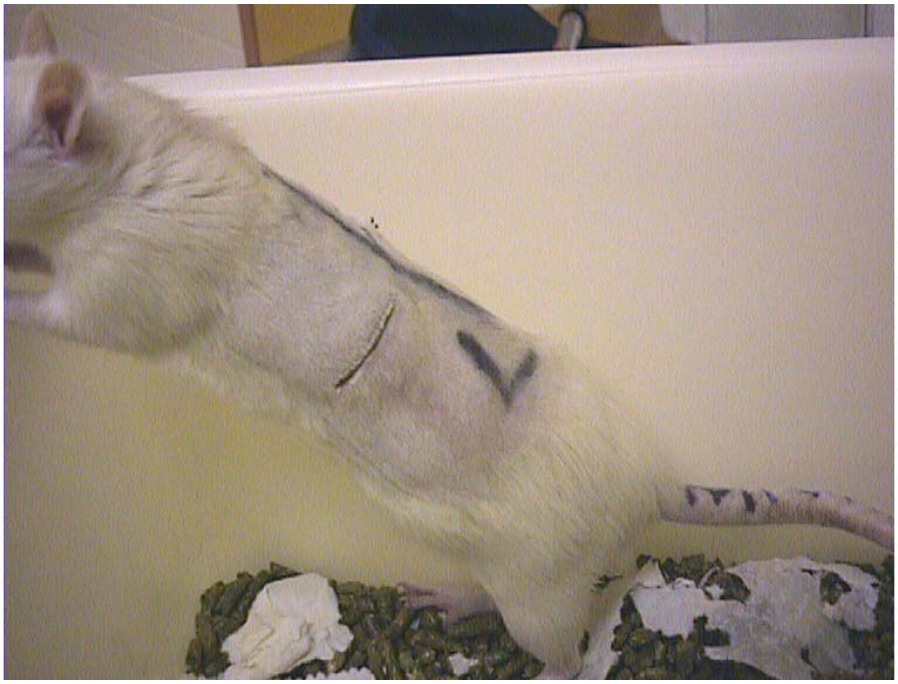


Figure 6-32 Laser wound showing normal healing - day 2

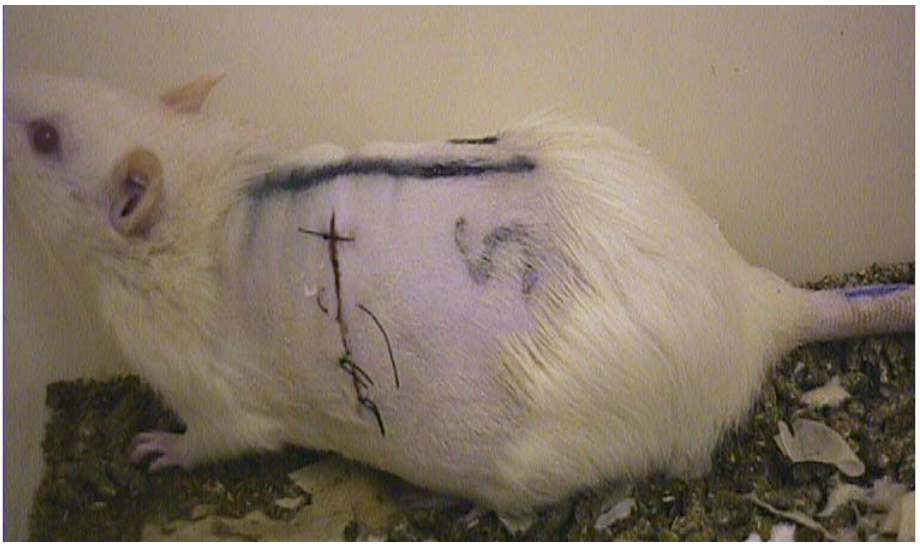


Figure 6-33 Normal healing of sutures, stitches undone by rats grooming

Time to achieve full strength equivalent to the baseline parameters was considerably longer than expected. Taylor el al (1997) stated a 3-week period was a sufficient time period for the collagen

responsible for tensile strength to be deposited. At this time however samples were found to have achieved less than half their uninjured strength.

Rats were due to be sacrificed at weekly intervals but when it became apparent that samples were not going to achieve full strength in this time frame the intervals between sacrificing become longer. Rats were sacrificed at day 7, 14, 21, 28, 35, 42, 60 and 91. The test at day 91 indicated that both laser and suture wounds had achieved the tensile strength indicated by the initial baseline tests.

Sutured wounds appeared to heal better in the early stages but this was not supported by the tensile tests where there is no significant difference in the yield force. Figure 6-36 show the laser and suture wounds at day 7. The sutured wound appears to have healed completely whilst the laser wound is still very obvious with a scab like crusting on the surface. The underside of the skin shows the deep tissue effect caused by the laser. This deep tissue effect had no long term repercussions as shown by the histological analysis.

Figure 6-34 Normal healing of wounds - Laser

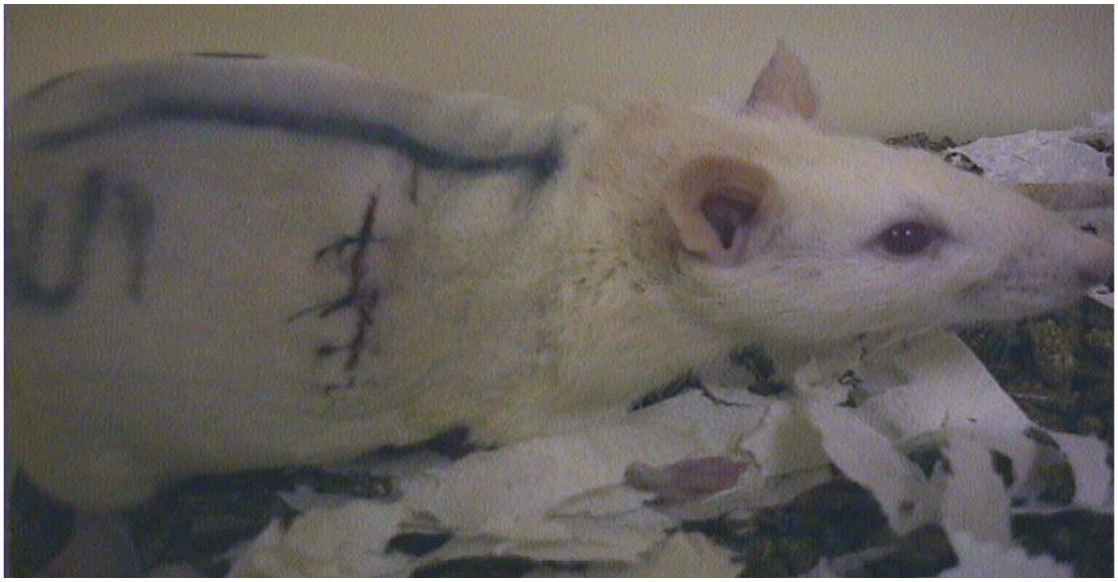


Figure 6-35 Normal healing of wounds - Suture

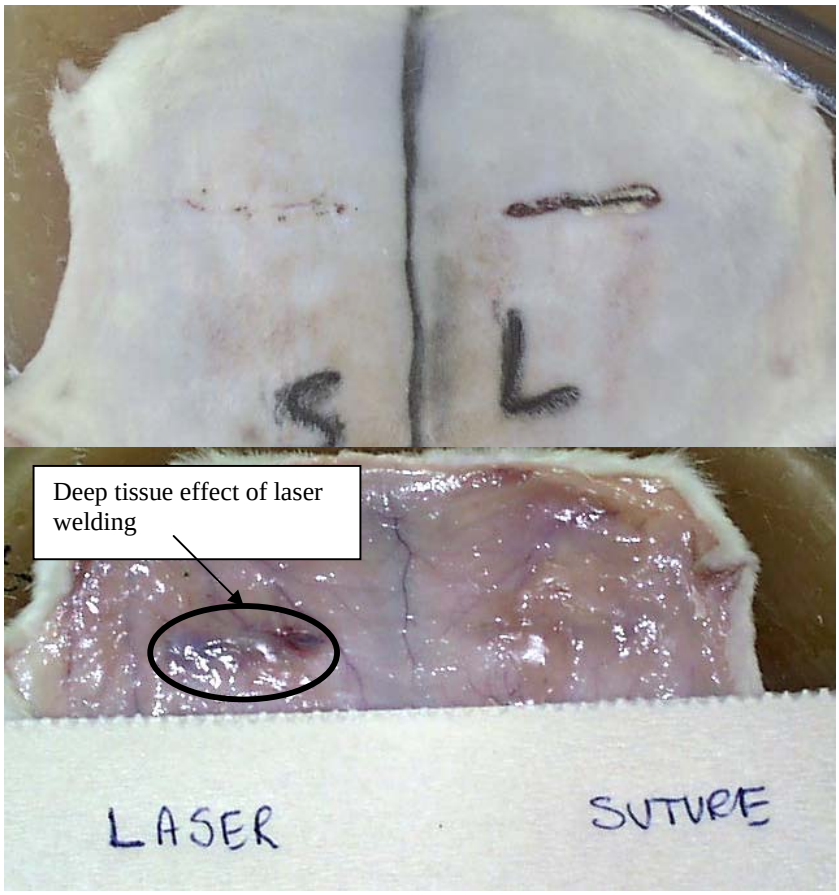


Figure 6-36 Rat 3-4 at 7 days (a) epidermis (b) dermis

The healing rate data for suture and laser wounds are shown in Figure 6-37 and Figure 6-38. Skin wound healing in rats may be affected by age, body weight, estrus cycle, room temperature, nutrition, activity, and hair growth cycle (Taylor et al, 1997). The animals in this study were matched for age, sex and body weight. They were fed the same diet and housed under the same conditions in order to remove these confounding variables from the analysis.

Most published literature on tensile testing of skin and wound healing rates give results in kg/cm^2 or kg/mm^2. Such measurements have little use with no corresponding information on skin measurement techniques, data or possible errors e.g. subcutaneous fat, connective tissue and hair. Therefore a direct comparison with results from this project is not practical. Results published by Levenson et al (1965) show similar trends in healing rates and times to those recorded in this project.

Simhon et al (2001) reports the tensile strength for sutured and laser *soldered*[7] wounds over a 28 day period. The tensile strength is reported in Newtons. Rats were sacrificed at 3, 7, 14 and 28 days. These results show the same trend and are very similar results to these in this project. They report a tensile strength for sutured wounds of 0.6 ± 0.4N at day 3 improving to approximately 11 N at day 28. This compares favourably with these results of 1.4 ± 0.25 N at day 7 and 8.05 ± 1.2 N at day 28. (Figure 6-37)

The laser healing rates follow a similar trend to that of sutured wounds. At day 7 the tensile strength was 1.3 ± 0.8N. At day 28 the tensile strength was 9.55 ± 2.0 N. (Figure 6-38)

[7] Laser soldering is applying a biological solder onto the approximated edges of the wound and heating the solder and underlying tissue with laser (CO_2 free beam) (Simhon et al, 2001).

Both laser and suture samples had reached half strength by day 42. Saunders and Reinisch (2000) reported a time to reach half strength for scalpel incisions of 24.5 days. This is significantly less than this study. Buell and Schuller (1983) indicated a time to half strength for scalpel wounds of approximately 42 days but indicated that wound had returned to full strength by approximately 56 days. In both cases similar trends in healing time and resulting tensile strength were reported.

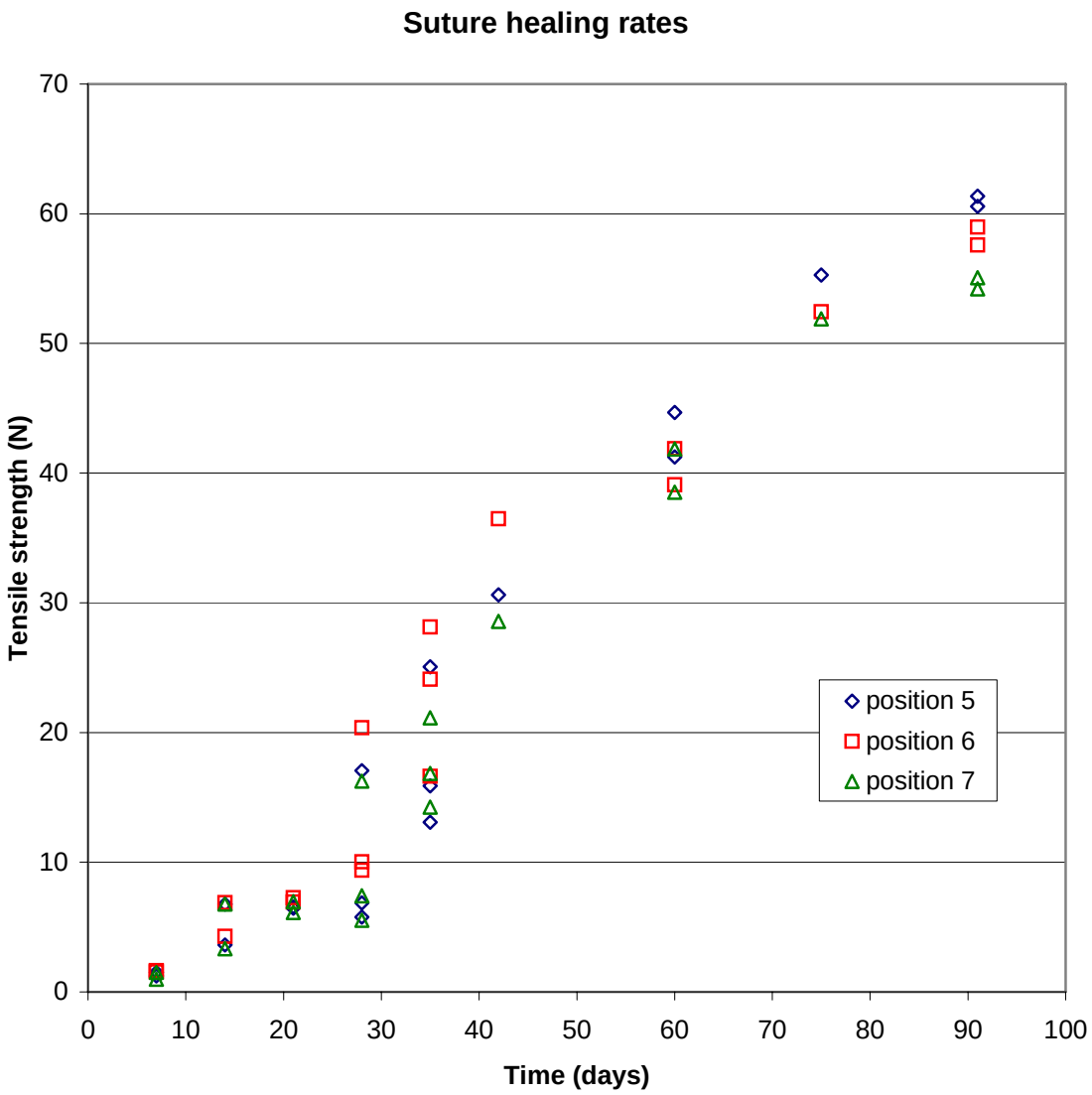


Figure 6-37 Tensile test of suture wound showing healing rates

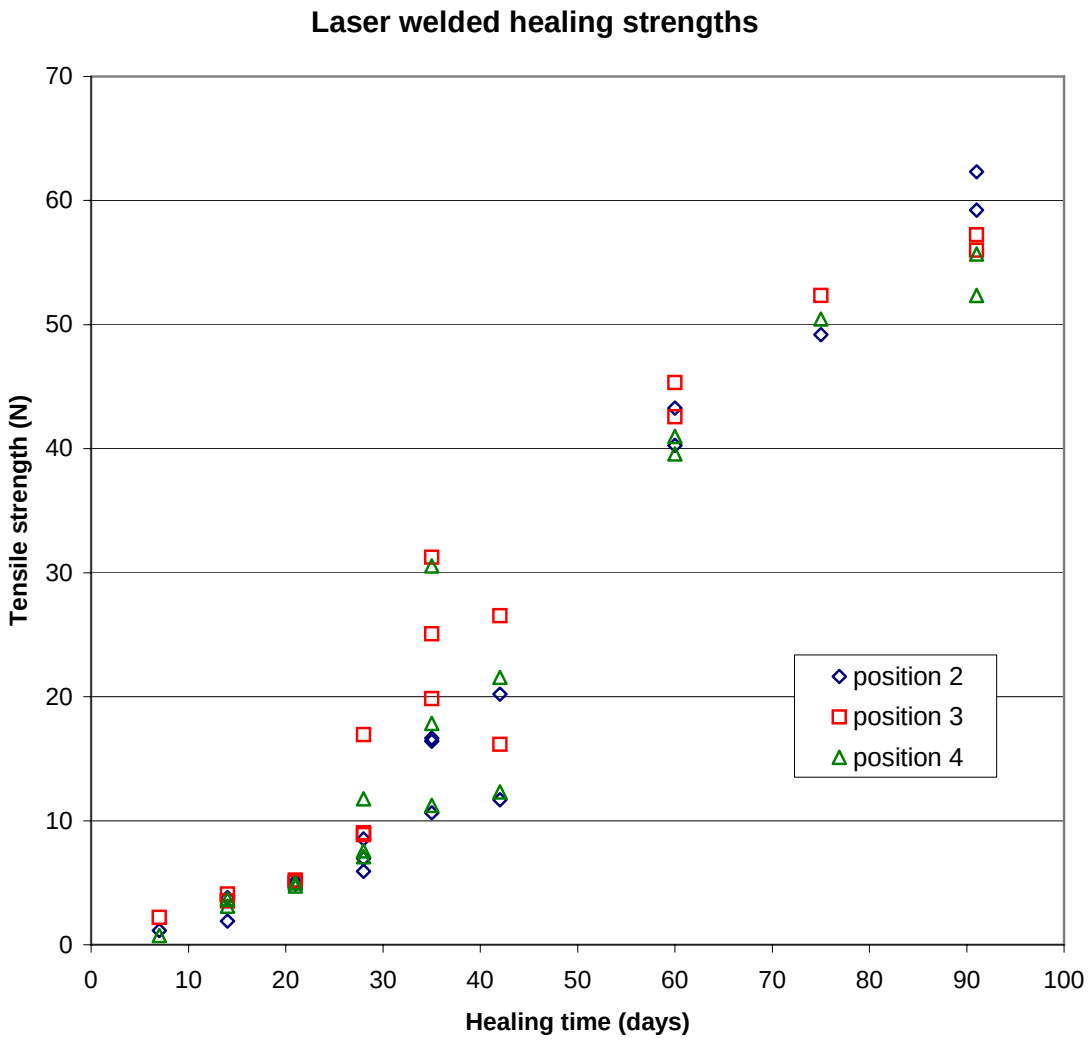


Figure 6-38 Tensile test of laser welded wound showing healing rates

6.5 Statistical model for laser welded and suture healing

6.5.1 Laser Welded Wounds

A linear regression model was calculated for the strength of the laser welded wounds with respect to healing time. This final equation for this regression is shown in **Equation 6-1**.

$$S = -7.78 + 0.709\ T \qquad \textbf{Equation 6-1}$$

Where:

S = strength of the wound

T = healing time

This linear model is significant at the P= 0.05 level and accounts for 88% of the data variability. The model also shows that age and position of sample are not significant.

Table 6-7 Model summary for laser welded wounds

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.940(a)	.884	.882	5.25455

a Predictors: (Constant), healing time

6.5.2 Sutured Wounds

A linear regression model was calculated for the strength of the sutured wounds with respect to healing time. This final equation for this regression is shown in Equation 6-2

$$S = -6.17 + 0.710\ T \qquad \textbf{Equation 6-2}$$

Where:

S = strength of the wound

T = healing time

This linear model is significant at the P= 0.05 level and accounts for 87% of the data variability as shown in Table 6-8

Table 6-8 Model summary for sutured wounds

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.931(a)	.866	.863	5.87854

a Predictors: (Constant), healing time

Equation 6-1 and 6-2 show the similarity in the data between the yield forces for sutured and laser welded wounds. They have a similar slope (0.71) and close intercepts.

6.6 A comparison of laser and suture strengths

A comparison was made between the strength of the sutured and the lasered wounds from twenty rats. Figure 6-39 shows a plot of the growth in mean strength for these two treatments. Data points represent the mean value of the replicated tests. The line shows a third order polynomial regression around these points for each treatment. At least 97% of the variation in the mean values is explained by regression.

The regression lines show that the sutured wounds healed slightly faster that the lasered ones. However this slight difference could be due to thermal damage of the tissue as Saunders and Reinisch (2000) reported delays in healing of laser **incisions** compared to scalpel wounds. They concluded laser incisions from a pulsed and CW CO_2 laser resulted in delays in healing of 1 day and 3.2 days respectively. It appears that even minimal lateral thermal damage can contribute to slower healing rates. Massicotte et al (1998) and Fung et al (1999) concluded that wound strengths are highly dependent on the tissue temperature during laser photocoagulation which relates to the degree of thermally induce tissue injury. However both treatments result in the same level of healing after 90 days. The final strength is comparable to the base line values for female rats at the midline position.

The two healing curves appear different but the variation in the mean data is within the naturally occurring variations in the strength of rat skin. The baseline tests discussed in Section 6.1 show that a female rat skin on the midline position has a standard deviation of approximately 15 N. The two curves on Figure 6-39 lie within this range of variability. It is concluded that the healing behaviour of laser-welded skin is not significantly different to that of sutured skin wounds.

Simhon et al (2001) comparing laser wound closure with albumin solder and CO_2 laser (free beam) with sutures also found no significant differences in the tensile test results to 28 days.

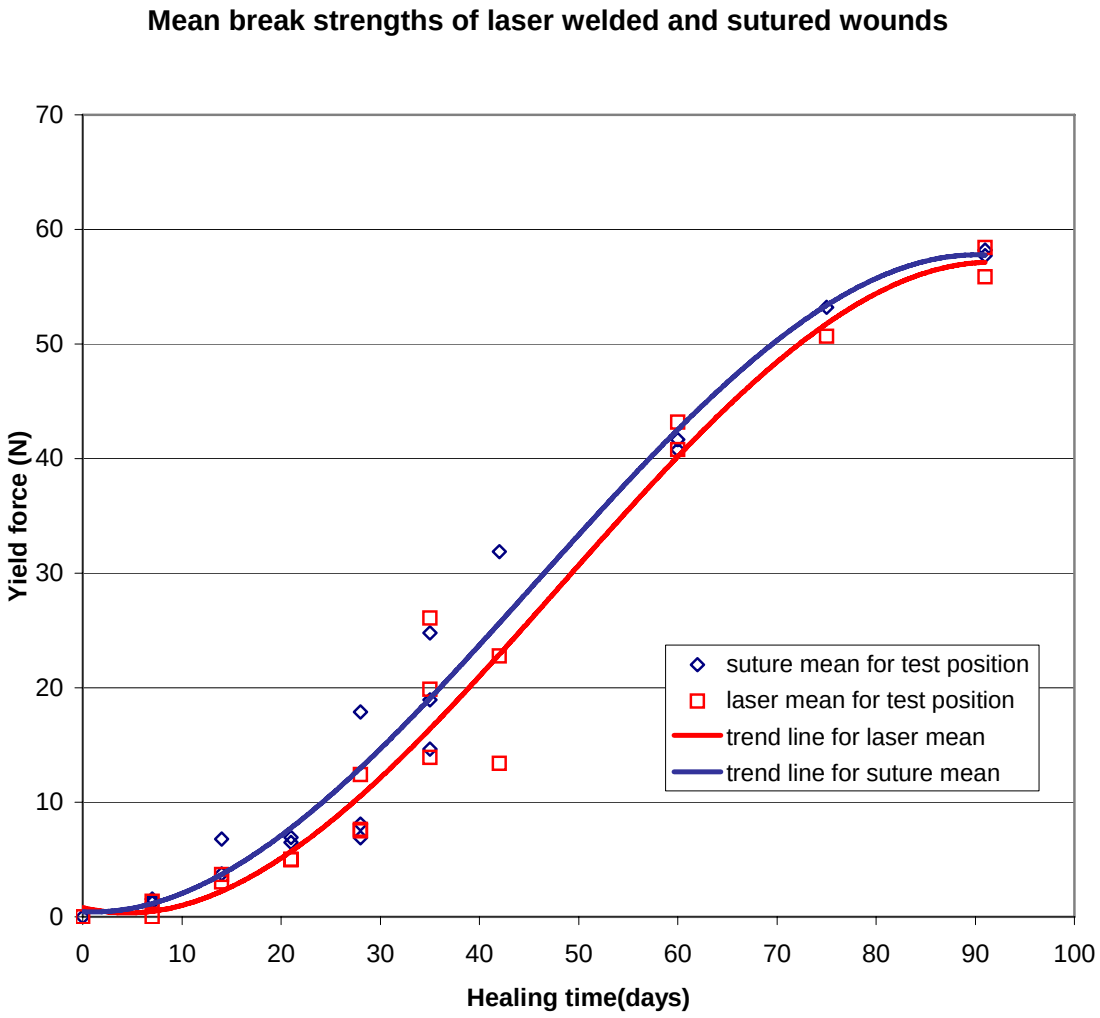


Figure 6-39 Laser and sutured healing rates

The results from the wound treatments were used to develop an understanding of relative healing behaviour following lasering and suturing.

Simhon et al (2001) published that a tensile strength of 0.6 ±0.4N was sufficient to hold tissue together. The first tensile test results for this experimental program is for day 7. It was found that the lasered wounds had a tensile strength of approximately twice this strength at 1.4 ± 0.5N.

It is concluded that laser welding using bipolar contact probes and the technique developed provides viable wound closure and comparable healing rates to suturing.

6.7 Histology

Samples from the laser-welded wounds were sent for histological analysis at Queensland University of Technology. Dr Brain Harmon undertook the cross sectioning, mounting and staining and supplied the light micrographs following. Dr Deb Stenzel has provided the histological analysis and assistance on its interpretation.

The samples were taken from the wound areas, shown schematically in Figure 6-40. These were then mounted on card and placed in a phosphate buffered formalin (10%) solution. Samples were embedded in paraffin and cross sections of approximately 5 microns in thickness were cut and stained with haematoxylin and eosin. These were examined for changes in cell composition and structure to determine cell damage and healing. As the samples were taken from the same area of the animal it was expected that all characteristics should be similar e.g. thickness of epidermis.

Complete analysis of the histology of healing would have required a different methodology as from a histological perspective the most interesting changes occur very early in the healing of the wound. The earliest samples sent for analysis were from day 7. At this point scalpel wounds visually appeared healed and laser welded wounds had a crusty scab in the epidermis. Both had only acquired approximately 3 % of the strength of uninjured skin.

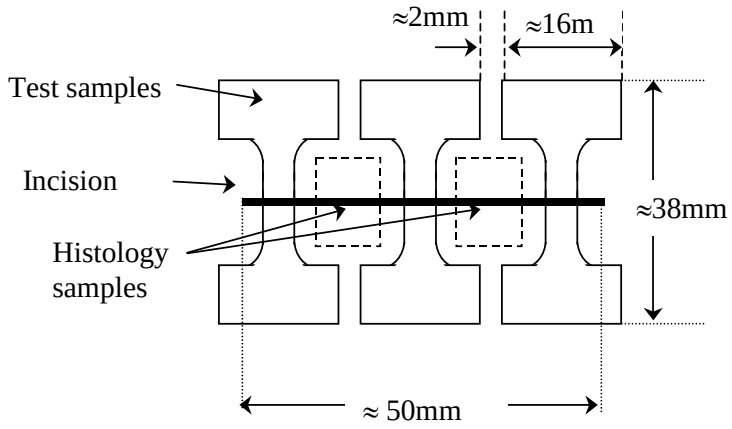


Figure 6-40 Samples for histological analysis

Light micrographs of several of the samples were taken and are shown in the following figures. The magnification rates are shown in brackets at the end of each figure title.

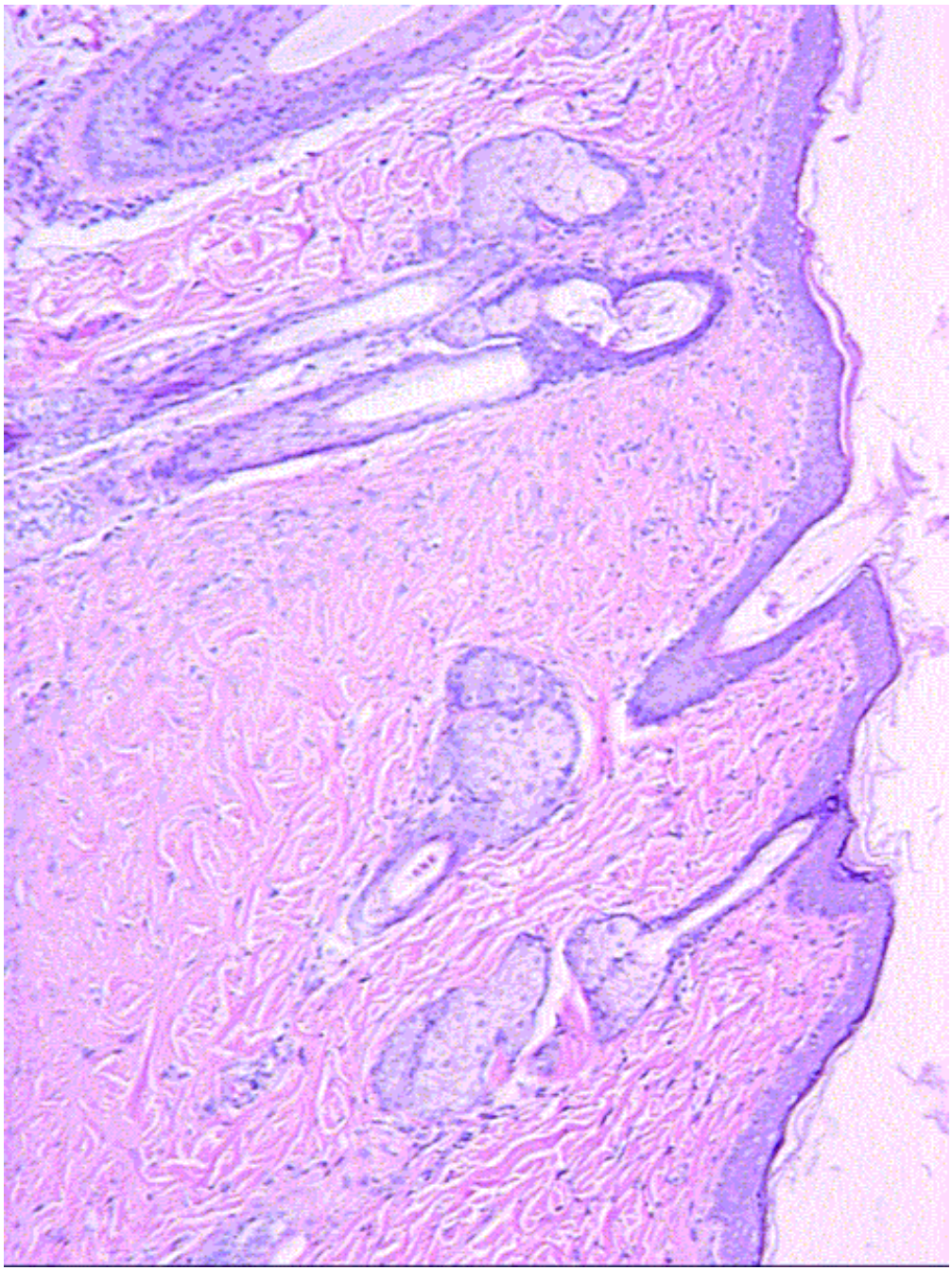


Figure 6-41 Cross-section of normal skin (X250)

Figure 6-41 is a cross section of normal skin for comparison with other histological slides. The slide indicates a thin epidermis often found in rodent skin.

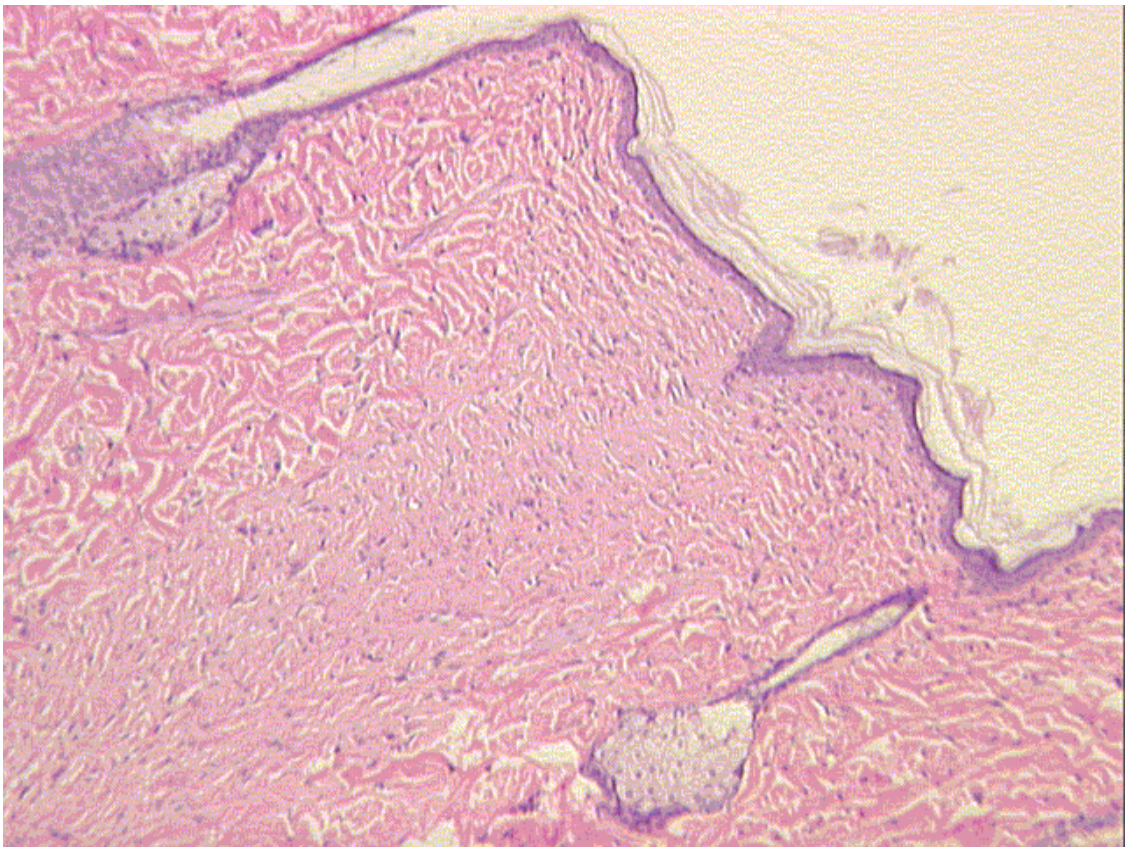


Figure 6-42 Low magnification showing scar tissue (X250)

Recent scar formation is shown by the pale fibroblastic tissue in the center of slide shown in Figure 6-42. The collagen bundles of the dermis differ from normal skin. The epidermis is intact and continuous indicating complete healing of the wound but it is thinner than normal over the lesion and shows some exfoliation of the outer layers. All samples were from the same region of the animal so the differing thickness may be due to very recent epidermal proliferation over the scar.

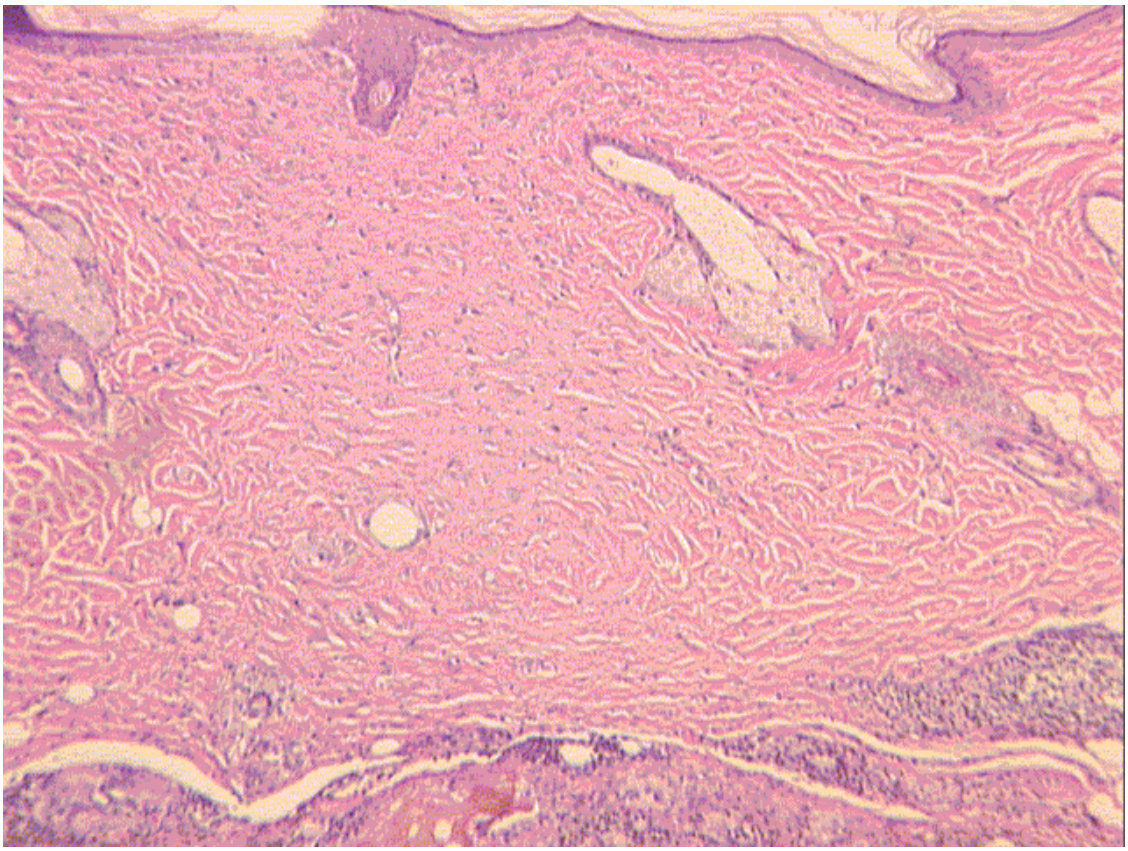


Figure 6-43 Hair follicle in area of healing (X250)

The healing is complete and less scar tissue is seen in Figure 6-43. The hair follicle is within the area of healing and the smaller amount of scar tissue indicates less lateral thermal damage. This supports the supposition that welds at lower temperatures reduce the formation of scar tissue whilst achieving equivalent healing rates. The welding technique and the bipolar forceps did not allow for consistent temperature profiles in the tissue.

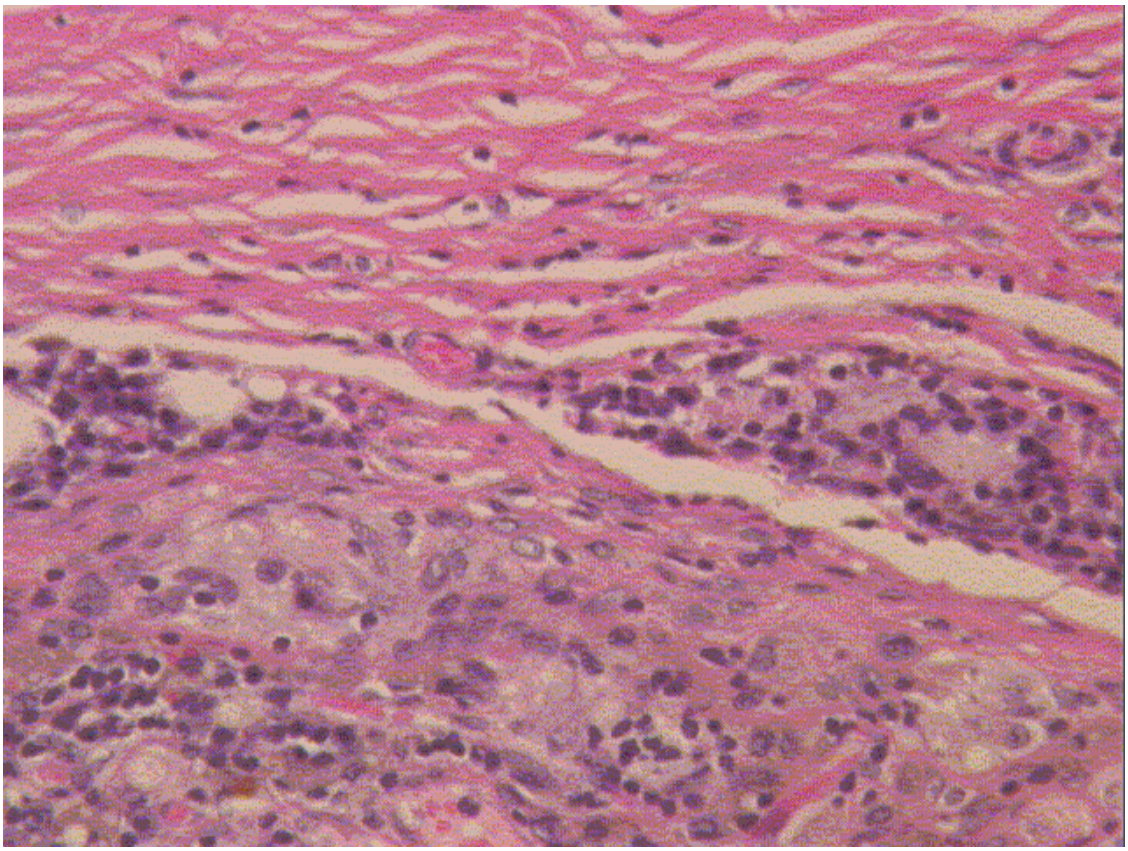


Figure 6-44 Subcutaneous tissues at high magnification (X1000)

The high magnification of subcutaneous tissues of Figure 6-44 indicates that the depth of thermal penetration did not reach this level or the damage was minor and healing has already occurred. Figure 6-36 indicated that there was deep tissue effect from the laser evident at day 7. However by day 14 (Figure 6-44) this effect is not present.

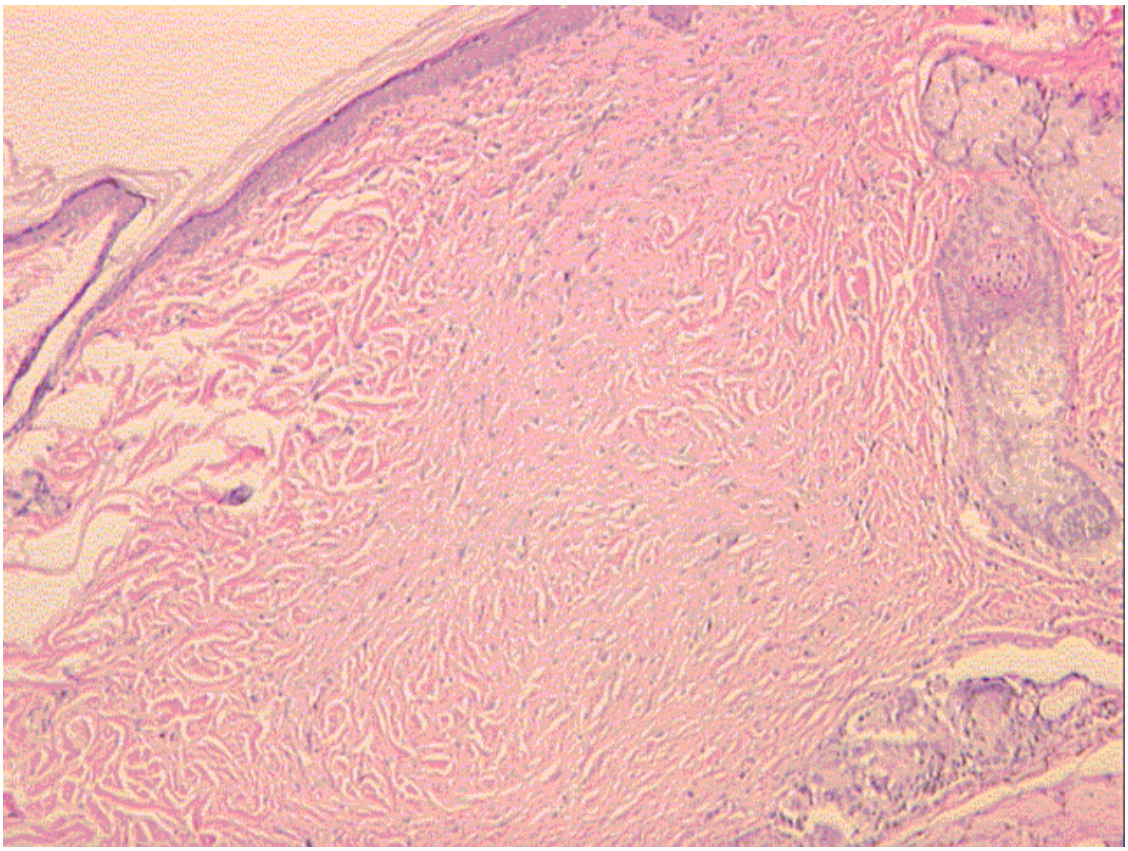


Figure 6-45 Fibroblastic scar tissue at low magnification (X250)

As the healing of the wound progresses the collagen fibres integrate throughout the cellular matrix giving the skin its increasing tensile strength. Figure 6-45 shows fibroblastic scar tissue and collagen fibres densely packed but without the normal orientation. This orientation of the fibres is essential for the strength of the wound. The wound appears fully healed but at 28 days is still only approximately 20% of full strength.

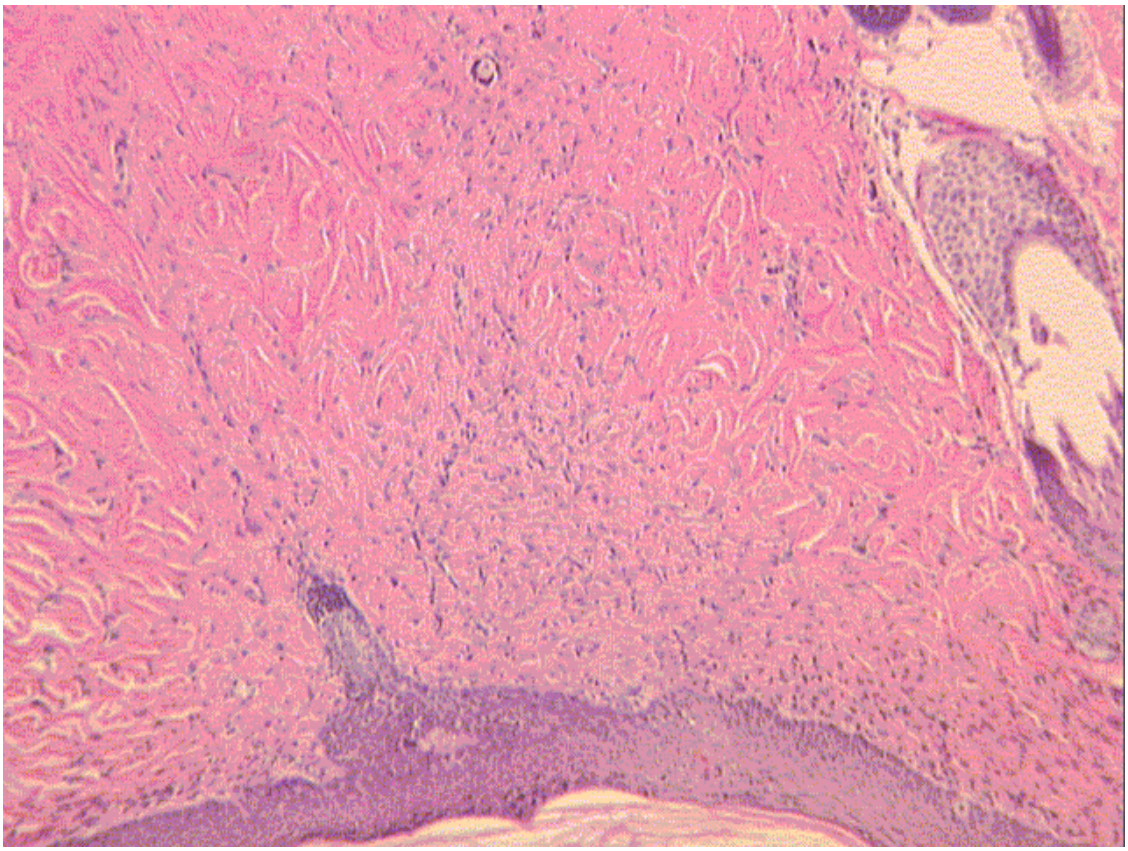


Figure 6-46 Dense fibroblastic scar tissue (X250)

The welding technique was a two stage process (refer to Figure 5-11) where the subcutaneous tissue and epidermis and dermis were welded in two separate stages as described in the methodology. This process can result in a significant increase in temperature of the upper layers due either to the thermal relaxation or thermal recovery times allowing the build up of excessive heat. Figure 6-46 indicates that this heat causes a thickening of the epidermis but no significant loss of wound strength.

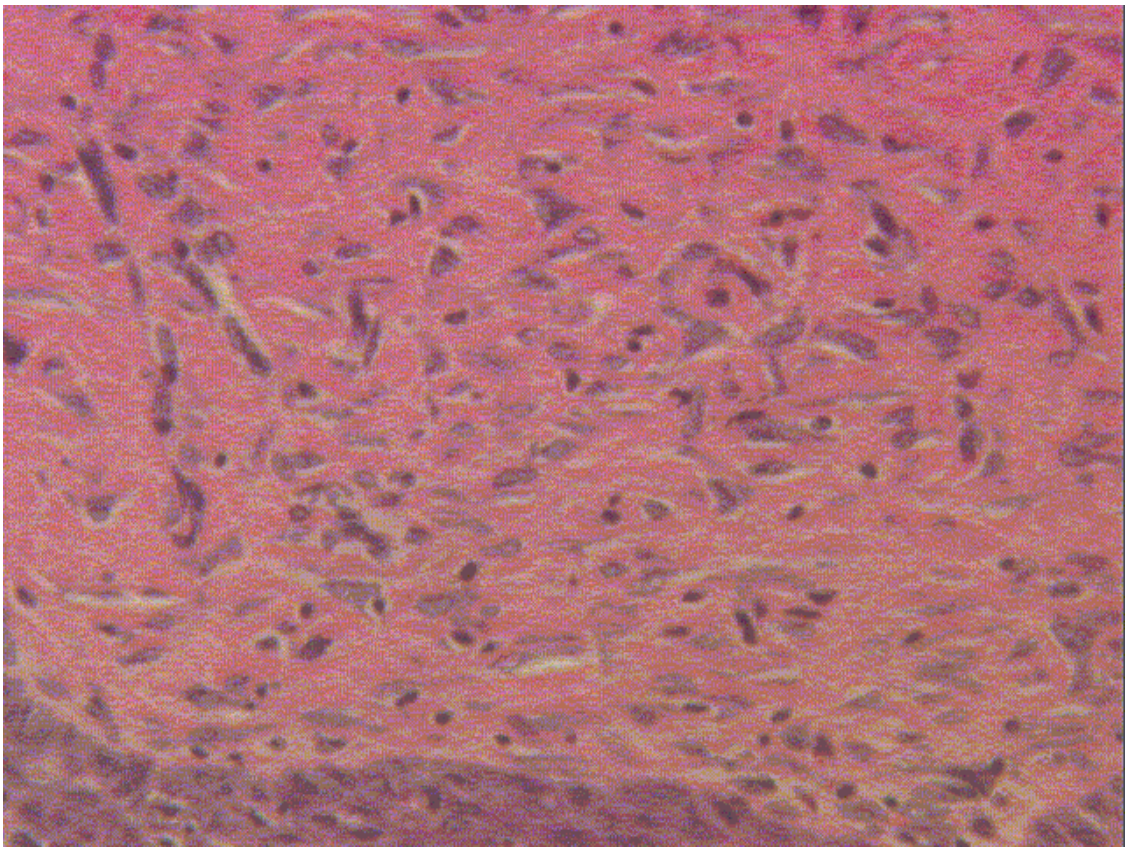


Figure 6-47 High magnification showing cellular nature of scar (X1000)

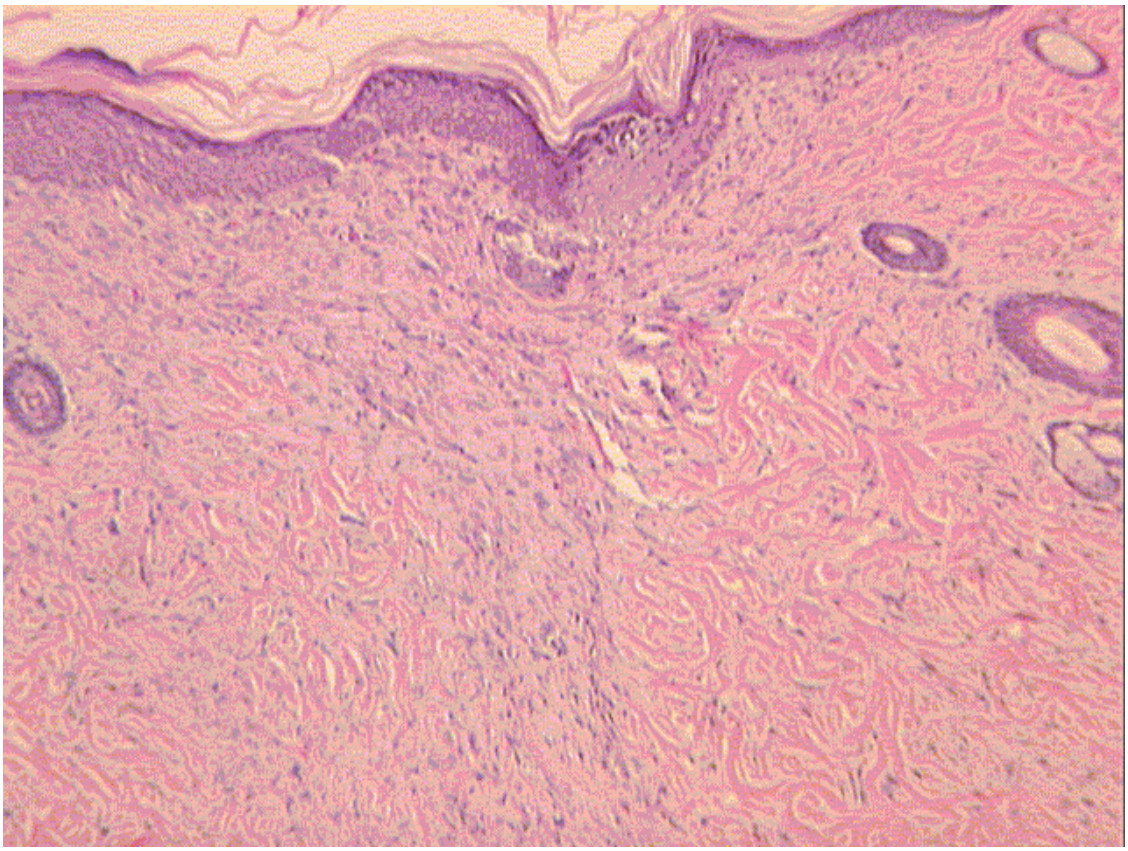


Figure 6-48 Low magnification of scar tissue (X250)

Visually it appeared that sutured wounds healed better in the early stages (not supported by tensile tests) and the laser welded wounds formed a scab like crust on the surface which had usually disappeared by day 14. The epidermal layers however, continued to exfoliate as shown in Figure 6-48. This image also shows the scar tissue consisting of integrated fibroblastic/cellular and collagenous matrices.

Figure 6-49 is a higher magnification of Figure 6-48 showing the cellularity of tissue at epidermal-dermal junction.

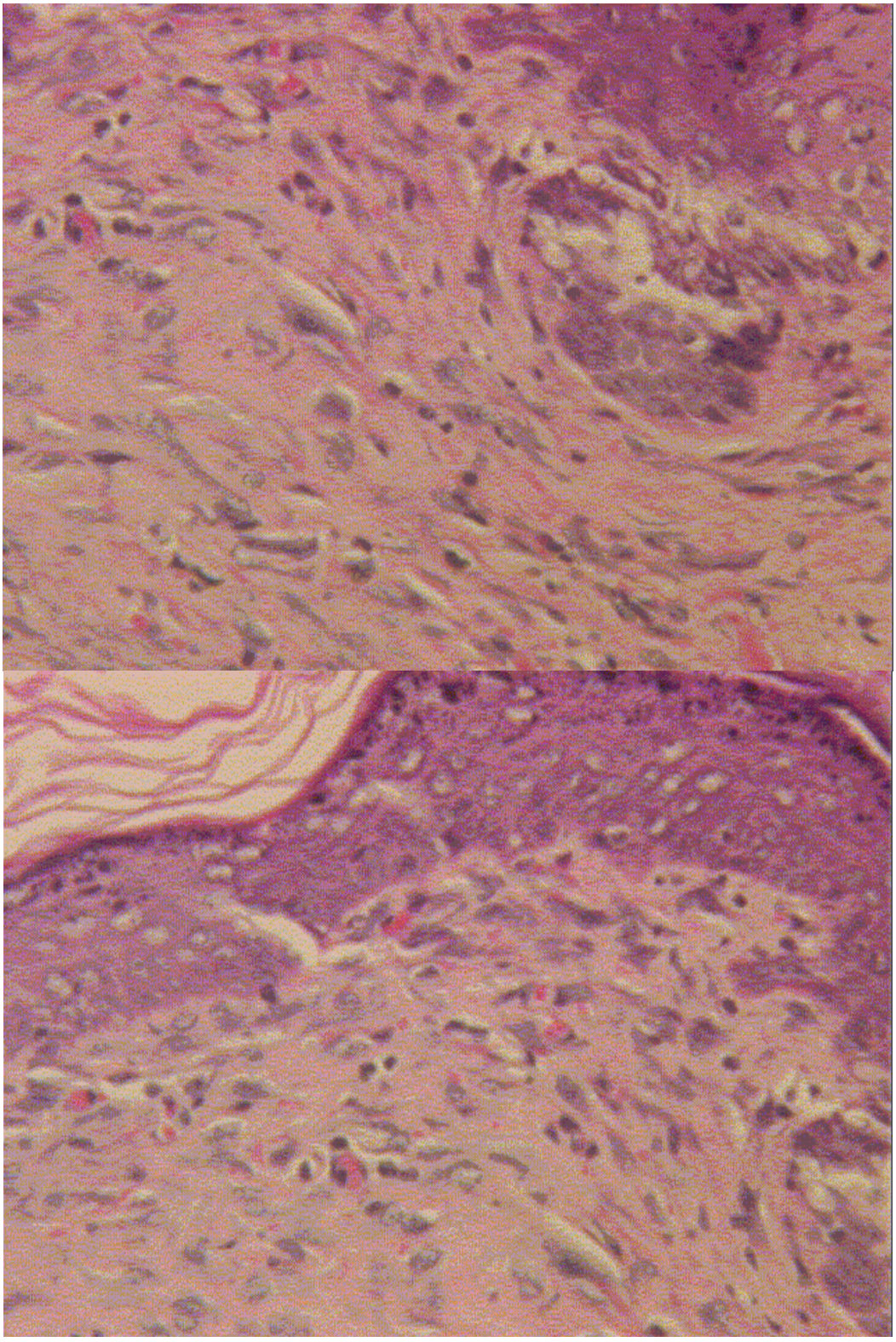


Figure 6-49 Tissue at epidermal-dermal junction (X1000)

7 Conclusion

The medical applications of lasers began in 1958 and several medical specialities have since benefited from laser technology. The applications have proven to be safe and cost effective. Lasers are currently employed in the fields of ophthalmology, gynaecology, otolaryngology, dentistry, and neurosurgery. In dermatology it is the preferred and in some cases the only tool available e.g. port wine stains and other hemangiomas. In ophthalmology the laser is the only instrument available and has supplanted traditional surgical approaches.

Nevertheless, lasers are not yet used to weld cutaneous tissue in clinical settings. The acceptance of any laser into the medical field for a specific clinical application is supported by a large number of experimental studies to investigate the appropriate wavelength, mode of operation and power settings. In some areas the findings can be, at best, inconclusive and at worst contradictory, as is the case of the effect with biostimulation by laser light to improve healing. In others the advantages offered by the laser are clear and obvious.

The rapid development of laser technology has made many lasers obsolete when only a few years ago they were considered state of the art. Investigations develop new treatment techniques and applications. Leading publications are quickly dated for example the article by Bailin PL et al (1990, page 161) quoted "As for yellow light therapy, no such potential is thought to exist for therapy of vascular lesions." Just 4 years later the Australian Institute of Health and Welfare, Canberra produced a government publication titled "Yellow light lasers in dermatology: Laser treatment of superficial cutaneous vascular lesions". This publication went on to summarise the use of yellow light lasers in the treatment of dermatological conditions.

There are few disadvantages to lasers in specific medical applications provided the type and mode of operation is correctly selected for the desired tissue effect. Lasers are mostly operated in the non-contact mode and this removes the tactile guidance traditional techniques offer. Medical schools have been slow to provide training in the techniques of laser surgery except in fields where the laser is the preferred or only tool (ophthalmology and dermatology). The high capital cost and that of ongoing support staff training together with implementing safety protocols and laser and applicator hardware maintenance make the laser a very expensive overhead which must be utilised regularly. These disadvantages lead the cynics to claim that the use of lasers by doctors, particularly in areas such as dermatology, as merely a marketing tool. Such cynicism overlooks the clear advantages in many areas proven by researchers over decades of investigation.

Researchers are continuously evaluating new lasers, laser tissue interaction, histology of radiated tissue and surgical and medical applications. Despite this effort by researchers to push the boundary of knowledge, laser therapy will not be accepted if alternative methods are available which offer a better rate of success, are less dangerous to the patient or are easier to perform. In general though many useful laser techniques have been developed and clinically established.

In the early 1980's a new field for the medical application of lasers emerged in the literature – laser bonding of tissues. In this area there have been two fundamental approaches, laser welding and laser soldering. The latter applies a biological solder to the edges of the tissue and then the application of heat from a laser to both the solder and the underling tissue. Tissue welding uses laser light to heat the approximated edges of the tissue (Simhon, 2001). Laser welding and soldering have been investigated using a variety of lasers whose wavelength have different penetration profiles and various solders and absorption increasing dyes. All these investigations

used laser light in the free beam mode. No published literature was found on the use of bipolar contact probes to weld cutaneous tissue.

It appears that the investigation and final uptake of lasers into the medical profession is hindered by the lack of appropriate *engineering* and not the willingness of investigators and professionals. Frank (1987) states laser radiation is only as useful as the available accessory instruments and it wasn't until the mid 1990's that temperature control with closed loop feedback was investigated for tissue welding. This is remarkable given that the temperature of the tissue is the controlling factor in the viability of the weld.

Both laser welding and laser soldering are very promising surgical techniques. Claimed advantages include:

- Faster and easier techniques than suturing.
- Less foreign body reactions (Bass and Treat, 1999).
- Formation of an immediate water-tight seal.
- Better cosmetic results (Wider et al, 1991 and Abergel et al, 1986).

The claims of faster wound healing are contradicted by some published results.

Fung et al (1999) investigated the effect of temperature on weld strength using a 1320 nm Nd:YAG laser and concluded that tissue temperatures of 95^0C produces a weld with the strongest initial strength whilst 65^0C had the best results after 14 days. Simhon et al (2001) used tissue temperatures of $69 + 7^0$ C but Neimz (1996) reports irreversible tissue effects if temperatures greater than 60^0C are achieved. All researches agree that the underlying laser tissue interaction

is photothermal and specifically, coagulation, but the exact welding mechanism is poorly understood, particularly where no additional material or solder is introduced into the wound.

To achieve a weld between two edges of tissue the zone of acute thermal damage needs to be limited as severally damaged cells need to be removed by the body before new cell growth and healing can take place. The required temperature should be sufficient to 'melt' or soften the collagen matrix to allow bonding and perhaps initiate apoptosis, which is thought to play an important part in wound healing.

This project investigated the use of Nd:YAG bipolar contact probes to weld cutaneous tissue. It was found that the laser, probes and parameters selected could provide a viable weld that was comparable to sutures.

The dominant parameter that affects the strength of the resultant weld was the temperature of the tissue. Despite the volume of work published on tissue welding and soldering there is little consistency in the exact temperature required to form a viable weld. An initial study into the effects of Nd:YAG laser and the resultant tissue temperature found that viable welds could be achieved with a temperature of approximately $55\ ^{0}$ C using bipolar contact probes. This temperature was significantly less than stated in several published papers however a desire to limit the thermal damage to the surrounding tissue prompted this decision. Scope for future experimental work would be the evaluation of tensile strength and healing time for different tissue temperatures. A detailed investigation into the tissue temperature profile would also be useful to future researchers and laser welding studies. The lower temperatures could also be attributed to the force on the aligned wound edges from the contact probes. This force was maintained during the thermal relaxation time of the tissue and may have attributed to the

formation of the weld. The effect of this force on both healing rates and weld strength could be developed in future investigations.

Known disadvantages of welding include technical difficulties with tissue alignment (McNally et al, 1999 and Bass and Treat, 1995), low tensile strength in the first few days (Poppas DP et al, 1996) and thermal damage due to poor temperature control (McNally et al, 1999; Small et al, 1998; Springer and Welch, 1993).

The welding techniques developed in this project overcome the first of these difficulties. The use of the bipolar laser probes substantially improves the ability to align the tissue edges to be joined. The probes give tactile feedback to the user and the pressure effect of the probes may assist with the welding process. The developed technique was no faster or easier than suturing.

The use of a contact method also meant that the welds were *spot* welds as opposed to a continuous weld that would have been achieved with a non-contact laser. Whilst the areas of welding were kept as close together as possible there were small sections which would not have been sufficiently heated simply due to the technique.

Viable welds and a useable technique for welding skin on rats were developed and tested. The resultant healing was comparable with published literature and both sutured and welded wounds returned to full strength as compared with the baseline data collected. All wounds had returned to full strength within 91 days. At 75 days there was not significant difference between laser welded and sutured wounds and they had achieved approximately 90% of full strength. Time to half strength was approximately 42 days and there was a larger standard deviation for both laser welded and sutured wounds. The most significant increase in strength and therefore healing occurred in the first 42 days. Simhon et al, 2001 states that a tensile strength of 0.6 N ± 0.4 N

was sufficient to hold tissue together. By day 7 the strength of the wound (laser welded) was more than twice the strength needed to maintain closure. There may have been sufficient healing for this to occur earlier but there were insufficient animals to allow for testing of this theory.

The published results of tensile tests for uninjured and healing skin was inconsistent and difficult to reproduce. Comparative analysis with these results is difficult because of inconsistency in units and lack of detail in the reporting of testing parameters and results. Test results are often given in non SI units e.g. kg/cm^2. The lack of exact data and results, measurement techniques, small sample numbers and relevant parameters (e.g. cross sectional area) made comparison of tensile strength of both base line tissue and welded wounds difficult to compare. Tensile strength is the force per unit area existing in a material prior to rupture. When critically assessing the strength of non-identical material, tensile strength provides a more valid comparison.

This experiment has found interesting trends in the tensile strength and elongation of rat skin. It used larger sample numbers than generally found in published literature and has reported them in an accepted scientific and engineering format. The methodology adopted a standard testing procedure and conforms to conventional practices. This will allow repeatability of the experimental procedure for verification and a continuation of the work.

The baseline tests showed that strongest yield force was recorded in test position 1 (spine and midline). All yield forces were symmetrical about the spine on the line of testing. There were large standard deviations due to natural individual variations in animals. The standard deviations were also symmetrical about the spine on the line of testing (i.e. head, middle or tail line). Experimental errors were minimised.

For female rats, the elongation of the test specimen at failure was also recorded. This indicated no significant difference (P= 0.05) between the elongation of the midline and tail positions. The elongation at the head position was significantly different, particularly at the edges of the rat skin (positions 18,17,20 and 21).

The elongation for test specimens for male rats was also recorded but due to small sample sizes no significant analysis could be undertaken.

Future work in this area is recommended to plot Langer's Lines. These lines indicate the direction of the collagenous bundles in the skin. The collagen in the skin is directly responsible for it's tensile properties (strength and elongation). The orientation of the collagen fibres either parallel or perpendicular to a wound may have direct consequences for the healing rate and strength.

One of the aims of this thesis was to investigate the healing rates and tensile strength of laser welded wounds and a comparison with sutured wounds. The methodology adopted to test this was not appropriate for a thorough histological analysis to investigate the effect of laser light and the welding technique at the cellular level. The histology undertaken did however show interesting changes including apoptosis of some cells, macrophage invasion and calcification (Harmon, 2000, personal communication).

The orientation of the collagen fibres and its integration into the cellular matrix has a clear effect on the tensile strength. Collagen is well integrated into the surrounding cells by day 7 but the tensile strength is approximately only 3%. At day 28 there are clear collagen bundles, the epidermis is complete over the wound but the tensile strength is only 15 % to 20% of full strength.

The tensile strength and healing rates have been investigated so a future investigation with a methodology to allow a full histological analysis would be appropriate. This could be integrated with tissue temperature profiles to provide definitive evidence on a specific welding temperature for bipolar contact probes.

In conclusion this experimental program and investigation has reviewed the available literature on the current use of lasers in medicine and their specific laser-tissue interaction which leads to tissue welding. It has provided a large database of tensile strength measurements collected with a reproducible methodology and reported in a standardised format. The developed technique for laser tissue welding using a bipolar contact Nd:YAG laser has been established and verified. It produces viable welds comparable in strength and healing rates to sutures.

Appendix A Basic Components and Operation of a Laser

Basic Components of a Laser

Laser light exhibits three unique properties that differentiate it from ordinary light e.g. from that a light bulb. Laser light is `monochromatic`, `directional` and `coherent` (see Glossary) and as the stimulated emission of light is much faster than spontaneous emission, the laser output can be powerful. It should also be noted that whilst the acronym laser stands for *light* amplification, the term is equally applied to devices producing radiation in the infrared and ultraviolet portions of the electromagnetic spectrum i.e. outside the visible spectrum. See Figure A-1.

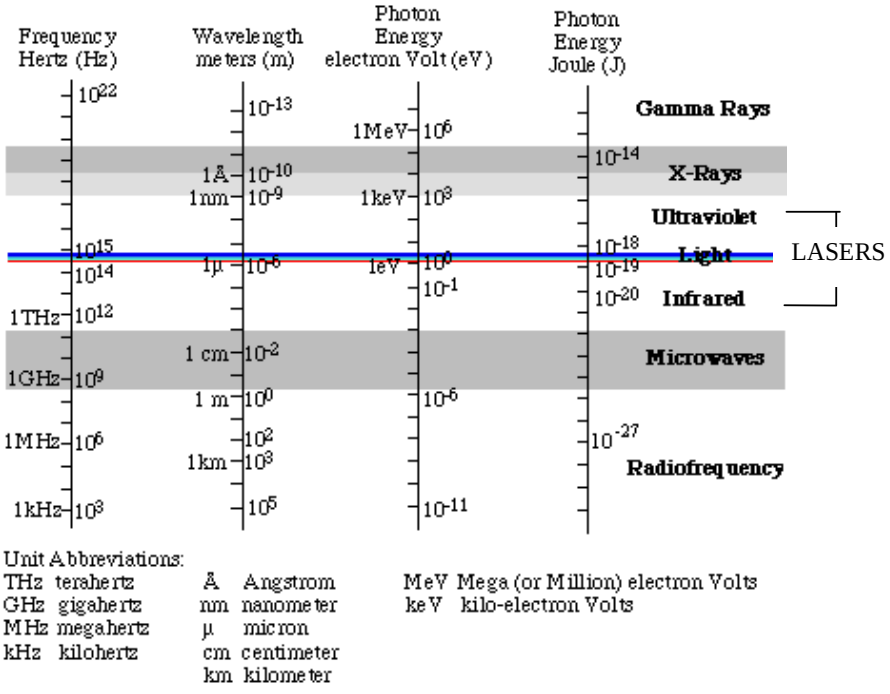


Figure A-1 Electromagnetic spectrum

(modified from http://science.nasa.gov/newhome/images/em.gif)

However regardless of the wavelength, power or mode of the output wave all lasers have three basic components consisting of a `lasing medium`, an `excitation mechanism` and a

`resonator` as seen in Figure A-2. The resonator is in turn comprised of a `feedback mechanism` and an `output coupler`. Descriptions of these components can be found in the glossary. Of these basic components it is the lasing medium which determines the frequency of the output beam and the four major types of lasing mediums are used for classifying lasers.

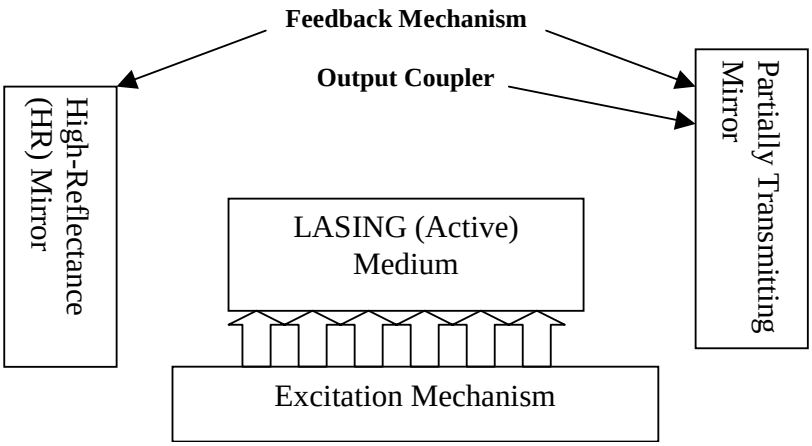


Figure A-2 Basic components of a laser

Basic Operation of a Laser

In the operation of a laser the excitation mechanism is a source of energy which excites (or pumps) the molecules, ions or atoms of the lasing medium into a higher energy state in order to create a population inversion. See Figure A-3.

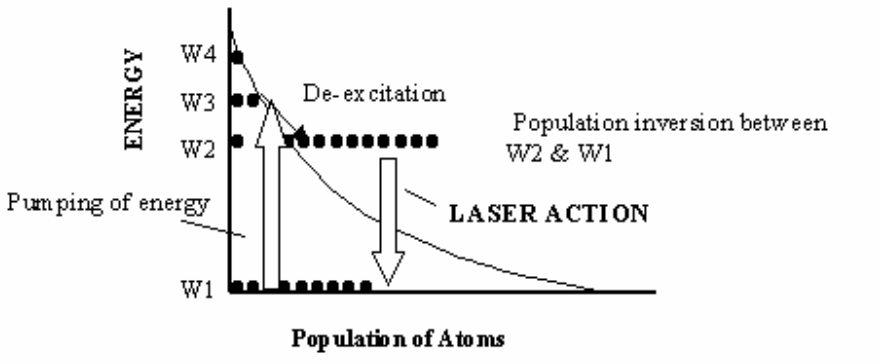


Figure A-3 Population inversion and pumping of atoms

This molecule then sheds this higher energy as a photon. Some of these photons that are emitted emit along the axis of the laser tube, the Fabry-Perot cavity which forms the feedback mechanism of the resonator. The photons bounce back and forth between the mirrors that are at either end of the laser tube. When an excited molecule is struck by a photon moving between the mirrors it is stimulated to emit a photon that is identical to the stimulating photon. This is the Stimulated Emission. The original and newly admitted photons continue to stimulate the production of more photons resulting in an Amplification of Light. Some of these photons are allowed to escape through a special transmissive mirror. Those that escape create the laser beam. See Figure A-4

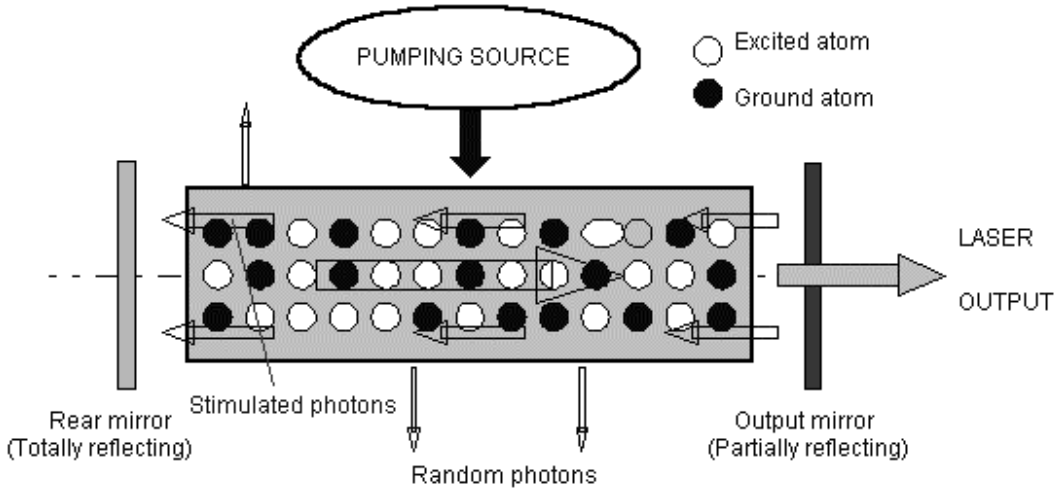


Figure A-4 Laser system

(http://vcs.abdn.ac.uk/ENGINEERING/lasers/optical.html , 9/11/1999)

Modes of operation

Lasers can operate in different modes which effect the power delivered to the target medium. There are two basic modes of operation, the continuous-wave (CW) mode in which the laser delivers energy constantly and the pulsed mode the laser delivers regular bursts of energy of very

short duration. The pulsed mode can be further specified by the length of the pulse and hence the method of pulsing the laser light. The first of these is the Q-switched mode. By employing the Q-Switch technique the output power of a pulsed laser can be significantly increased, with a corresponding decrease in the pulse duration. In this mode a shutter blocks the optical path between the lasing medium and one of the mirrors, resulting in a very high degree of excitation of the lasing medium but no laser action. When the shutter is opened, the energy is released, producing a pulse of very high energy and very short duration

Even more powerful and shorter pulses are obtained in a mode-locked laser. In an ordinary laser the oscillation takes place at different closely separated wavelengths. In a mode-locking technique, the different modes of oscillation are synchronised so that at a particular instant and for very short time the peaks of their waves coincide. This results in an intense short burst of energy.

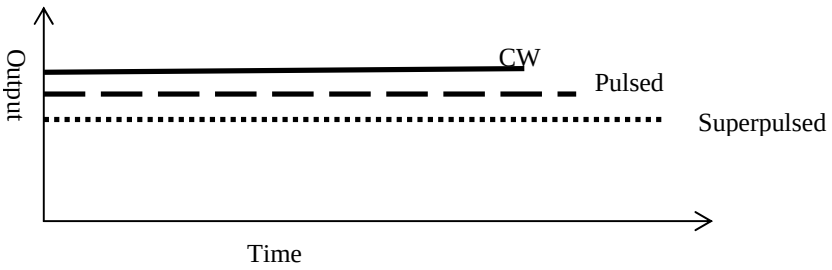


Figure A-5 Modes of operation

Chopped pulses with a duration in the millisecond range are obtained from CW lasers when using rotating apertures. Superpulses are achieved by modulation of the high voltage discharge. Thereby pulse durations less than 1ms can be generated. The peak power is inversely related to the pulse duration. The mean powers of the CW radiation and chopped pulses are nearly the same, whereas it decreases in the case of superpulses. As discussed in section 2.7.2 shorter pulse

durations are associated with a reduction of thermal effects. Hence, by choosing an appropriate mode of the laser, the best surgical result can be obtained.

Terms to describe the laser beam

Focal Length

A lens with a short focal length can provide a smaller spot size, thus increasing the intensity of the beam. To give a medical example a carbon dioxide laser with a 50mm lens can produce a spot size of 0.1mm, whereas a 400mm lens can produce a spot size of 0.8mm in size. Lenses can be modified to achieve spot sizes in the range from 0.025 to 0.05mm for very precise surgeries, such as in laser welding or ophthalmological applications (Ball 1992).

Wavelength

The wavelength of the laser also limits the spot size and beam focusing. With all other factors being equal, shorter wavelength can generate smaller spots. Thus, an argon laser at 488 nm can produce a much smaller sport than the CO_2 laser at 10 600 nm. The choice of laser should be made according to the specific effects of the laser on the tissue rather than based on its spot size capabilities.

Transverse Electromagnetic Mode

A beam of light propagating down the optic axis of the laser will be amplified and, provided sufficient gain is available, will emerge from the laser output mirror. Generally speaking, a photo travelling at right angles to the optic axis will not be amplified for form a laser beam as, although it may cause many stimulated emissions, there will be an insufficient number to overcome all losses.

However in the situation where a wave is travelling only slightly off axis the wave may be able to 'zig-zag' between the mirrors a sufficient number of timers to produce enough gain to overcome the losses. The result of this is to produce an output which may consist of a variety of complicated patterns of light.

These patterns are the result of the laser operating in what is referred to as transverse modes. Such modes have well known equivalents in microwaves and are designated by the same notation as $\mathbf{TEM_{pq}}$ modes. The laser output may consist of a mixture of different transverse modes and figure x.x indicates how the modes are labelled.

The TEM mode determines the precision of the spot by the power distribution over the spot area. A TEM_{01} mode is an example of a common multimode distribution, meaning that the sport has a cool area in the centre of the beam. The most fundamental mode is the TEM_{00}, which produces a Gaussian intensity distribution over the spot. Thus most of the power is concentrated in the centre and the rest decreasing in intensity towards the periphery of the beam. The spot size of a TEM 00 beam is the region that has approximately 86% of the total beam power. This mode produces the smallest precise spots (Ball, 1992 p 10) .

Energy

As output beam of the laser has many different applications the main terms used to describe the beam, apart from the characteristic wavelength are the *radiant power* expressed in watts (W); *energy* expressed as joules (J); *fluence* laser energy density expressed as joules/cm^2 and *irradiance* or power density expressed as Watts/cm^2.

The power of a laser is measured in watts (joules/second). The single most important factor in the effective application of the laser the *power density* or *irradiance*. Power density is defined as the amount of power that is concentrated into a spot, or watts/cm^2. Thus a larger spot size will allow laser power to be spread over a greater area, thus decreasing the intensity of the beam. Refer Figure A-6.

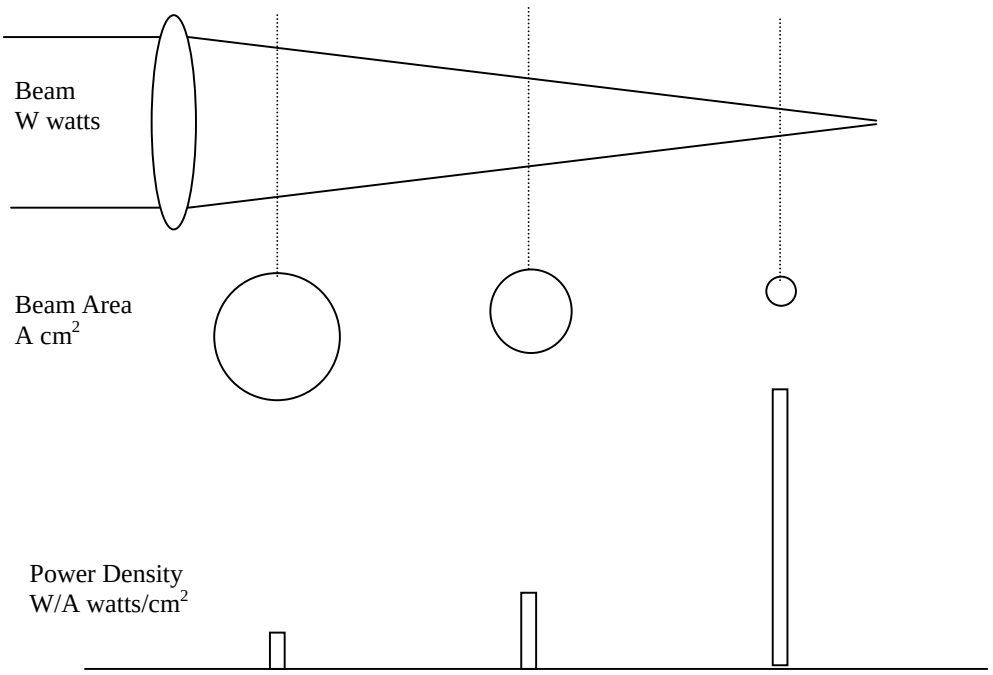


Figure A-6 Variation of power density with beam area

The spot size of the laser beam depends on several variables, including the focal length of the lens, the wavelength of the laser, and the transverse electromagnetic mode (TEM).

Fluenece is the laser *energy density* expressed as joules/cm^2. It is a concept involving the power of the beam and the length of exposure. It is the amount of energy that is delivered to the target area.

Appendix B Classification of Lasers and Laser Safety

Table B-1 Laser classification overview

Class	Definition
Class 1	The least-hazardous class. Considered incapable of providing damaging levels of laser emissions.
Class 2a	Visible Light lasers viewed for a duration of less than or equal to 1000 seconds
Class 2	Applies only to visible laser emissions and may be viewed directly for time periods of less than or equal to 0.25 seconds, which is the aversion response time.
Class 3a	Dangerous under direct or reflected vision. These lasers are restricted to the visible electromagnetic spectrum.
Class 3b	May extend across the whole electromagnetic spectrum and are hazardous when viewed intrabeam.
Class 4	The highest-energy class of lasers, also extending across the electromagnetic spectrum. This class of laser presents significant fire, skin, and eye hazards.

Class I

Class I lasers are lasers which cannot cause injury from viewing the *accessible* laser radiation for the maximum possible duration inherent in the design. Very few lasers are Class I, however, many laser systems can be made into Class I systems by totally enclosing the laser beam and interlocking the enclosure. Thus they are designed in such a way that prevents human access to the laser radiation. These consist of low power lasers and of higher power *embedded* lasers. Laser printers (embedded) and bar code scanners (low power) are in this class. Other examples of Class I laser use are: CD players, CD ROM devices, geological survey equipment and laboratory analytical equipment.

No individual, regardless of exposure conditions to the eyes or skin, would be expected to be injured by a Class I laser. No safety requirements are needed to use Class I laser devices.

Class IIa and Class II

Class IIa lasers are defined as visible lasers that have a wavelength of between 400 nm to 700 nm. They are not intended to be viewed and do not exceed the Class I AEL (Accessible Emission Limit) for an exposure duration less than or equal to 1000 seconds.

Class II lasers are low power (may not exceed an output power of 1 mW), visible light lasers that could possibly cause damage to a person's eyes if viewed for long periods of time. They are considered safe for general use because the *human aversion response* (see Glossary and section 0). Eye injury can occur if collecting optics are used in viewing the beam or if an individual overrides the aversion response and continues to stare into the beam path.

Class IIIa and Class IIIb

Class III lasers are defined as lasers which may cause injury through intrabeam viewing or through viewing a `specular reflection` for less than 0.25 second. Viewing a `diffuse reflection` from a Class III laser should not cause injury to the eye. This class is split into two smaller classes Class IIIa and IIIb.

Class IIIa lasers are continuous wave, intermediate power (1-5 mW) devices. They are defined as an invisible laser with an output power which does not exceed 5 times the Class I AEL or a visible laser with an output power which does not exceed 5 mW. Some popular applications of lasers such as laser pointers and laser scanners can use either Class II or class IIIa lasers. Direct viewing of the Class IIIa laser beam could be hazardous to the eyes.

Class IIIb lasers exceed the output power of Class IIIa lasers but cannot exceed the upper power limit of 500 mW (intermediate power:- c.w. 5-500 mW or pulsed 10 J/cm^2). Some examples of Class IIIb laser uses are spectrometry, stereolithography, and entertainment light shows. Direct viewing of the Class IIIb laser beam is hazardous to the eye and diffuse reflections of the beam can also be hazardous to the eye.

Class IV

Most medical lasers are in this class. These can cause serious eye and skin injury and set fire to many materials. Both the direct and reflected beams are hazardous to the eye.

Class IV lasers are high power (c.w. >500mW or pulsed >10J/cm²) devices. Some examples of Class IV laser use are surgery, research, drilling, cutting, welding, and micro-machining. The direct beam and diffuse reflections from Class IV lasers are hazardous to the eyes and skin. Class IV laser devices can also be a fire hazard depending on the reaction of the target when struck. Much greater controls are required to ensure the safe operation of this class of laser devices. Whenever occupying a laser controlled area, correct eye protection is required. Most laser eye injuries occur from reflected beams of class IV laser light, so all reflective materials must be kept away from the beam (http://www.uky.edu/FiscalAffairs/Environmental/radiation/laser_fs.html, http://radsafe.berkeley.edu:5016/lsm498c.html#anchor1596222 , AS 2211/NZS 5821)

Table B-2 Selected occupational exposure limits (MPE's) for medical lasers

(Sliney, 1995, Table 1 , page 372)

Type of laser	**Wavelength**	**MPE (eye)**
Argon-fluoride	193 nm	3.0mJ/cm^2 over 8 hours
Xenon-chloride	308 nm	40 mJ/cm^2 over 8 hours
Argon	488, 514.5 nm	3.2 mW /cm^2 over 0.1 sec
Copper vapour	510, 578 nm	2.5 mW /cm^2 over 0.25 sec
Helium-neon	632.8 nm	1.8 mW /cm^2 over 1 sec
Gold vapour	628 nm	1.0 mW /cm^2 over 10 sec
Krypton	568, 647 nm	1.0 mW /cm^2 over 10 sec
Nd:YAG	1064 nm	5.0μJ/cm^2 for 1ns to 50μs No MPE for t<1ns, 5mW.cm^2 for 10 s
Nd:YAG	1334 nm	40μJ/cm^2 for 1ns to 50μs 40mW/cm^2 for 10s
Pulsed Nd:YAG	1440 nm	0.1 J/cm^2 for 1ns to 1ms
Pulsed holmium	2100 nm	0.1 J/cm^2 for 1ns to 1ms
CW holmium CO_2	2100 nm 10600 nm	100 mW/cm^2 for 10s to 8hours, limited area 10mW.cm^2 for most of body (skin)

Table B-3 Laser safety standards reference

(http://www.cohsci.com.au/safety/lasersafety.html as at 10/9/00)

Title	Number	Publish Date	Scope	Notes
Part 1 - Laser Safety Classifications	AS/NZS 2211	April 1997	Details requirements necessary to protect persons from radiation from laser products	Updated version of the 1991 Standard
Part 2 - Safety of Optical Fibre in Communications Systems	AS/NZS 2211	April 1997	Outlines the safe use of lasers in communications	-
Recommended practices for occupational eye protection	AS/NZS 1336	January 1997	Recommended practices for the protection of the eyes of persons at work. Guidance is given on the selection of eye protectors appropriate to the use of particular lasers	Approved by SA Occupational Health, Safety and Welfare ACT April 1995
Personal eye-protection - Eye-protectors for adjustment work on lasers and laser systems	EN 208	August 1994	Laser adjustment eye-protectors for use in adjustment work on lasers and laser systems	Referred to in AN/NZS 1336
Personal eye-protection - Filters and eye-protectors against laser radiation	EN 207	August 1994	Applies to higher power lasers and eye-protectors against laser radiation than EN 208	Referred to in AN/NZS 1336
Guide to the safe use of lasers in health care	AS/NZS 4173	February 1994	Provides a guide to the safe use of lasers and laser systems in diagnostic and therapeutic medical and dental procedures	-
Guide to the safe use of lasers in the construction industry	AS 2397	1993	Guide to the safe use of lasers in the construction industry	-
Laser Safety	AS 2211	September 1991	Requirements necessary to protect persons from radiation from laser products	Approved by SA Occupational Health, Safety and Welfare ACT April 1995

This table of Laser Classification is intended as a guide, for complete details refer to the appropriate Section of their Standard or other related Standards

Table B-4 Laser safety requirements

(AS/NZS 4173:1994, page 14)

Laser Classification	**Potential Hazard**	**Installation**	**Personal**	**Training**
Class of laser not identified on machine or listed below	Do not use laser. Potential hazard level unknown. Laser unclassified. Contact supplier/manufacturer for classification and label. Laser does not comply with Australian, New Zealand or International Standards			
Class 1	Intrinsically safe refer section 5, Hazards	Nil	Nil	Operation only
Class 2	Low refer section 5, Hazards	Area warning signs	Do not stare in beam	Operation only
Class 3A	Low refer section 5, Hazards	Area warning signs	Do not stare in beam or view directly with optical instruments	Operation only
Class 3B	Medium refer section 5, Hazards	Area warning signs Controlled access Refer section 9 and Appendix A	Exposure to beam is dangerous, eye protection essential	Laser safety Operator training 'On-tissue effects' Refer Appendices D and E
Class 4	High refer section 5, Hazards	Area warning signs Controlled access Refer section 9 and Appendix A	Eye or skin exposure to direct or scattered radiation is dangerous, eye protection essential	Laser safety Operator training 'On-tissue effects' Refer Appendices D and E

Table B-5 Laser class with major hazards

(Modified from http://www.osha-slc.gov/SLTC/laserhazards/index.html)

Wavelength					**Hazards**		
Class	**UV**	**VIS**	**NIR**	**IR**	**Direct Ocular**	**Diffuse Ocular**	**Fire**
I	⎫	⎫	⎫	⎫	No	No	No
IIA	--	⎫*	--	--	Only after 1000 sec	No	No
II	--	⎫	--	--	Only after 0.25 sec	No	No
IIIA	⎫	⎫**	⎫	⎫	Yes	No	No
IIIB	⎫	⎫	⎫	⎫	Yes	Only when laser output is near Class IIIB limit of 0.5 Watt	No
IV	⎫	⎫	⎫	⎫	Yes	Yes	Yes
Key:	**UV** Ultraviolet **VIS** Visible Light **NIR** Near Infrared **IR** Infrared ⎫ Indicates class applies in wavelength range. * Class **IIA** applicable to lasers "not intended for viewing" *ONLY*. ** CDRH Standard assigns Class **IIIA** to visible wavelengths *ONLY*. ANSI Z 136.1 assigns Class IIIA to all wavelength ranges.						

Eye

Because of its special optical properties the eye is considered the most vulnerable to laser light. The Human Aversion Response is a natural protective instinct which protects the eye from visible light and infra-red wavelengths. This causes the lid reflex (blinking) to occur between 0.2 and 0.25 seconds, however this may not be fast enough to prevent injury from the intense light generated by a laser, and will not it protect from UV wavelengths.

Eye protection requires the understanding of two terms: `maximum permissible exposure` (MPE) (Table B-2 shows MPE levels for lasers) and `Nominal Hazard Zone` (NHZ). The NHZ is calculated using a laser range equation that considers the laser wavelength,

beam size and divergence, laser power, and range form the laser to the target. Diffuse reflection and scattering are also taken into account.

Damage to the eye falls mainly into two categories: damage to the retina by visible (400-760nm) and near infrared wavelengths (IRA: 760 - 1400 nm) and damage to the cornea by Far UV (UV-B) and infrared (IR-B, IR-C).

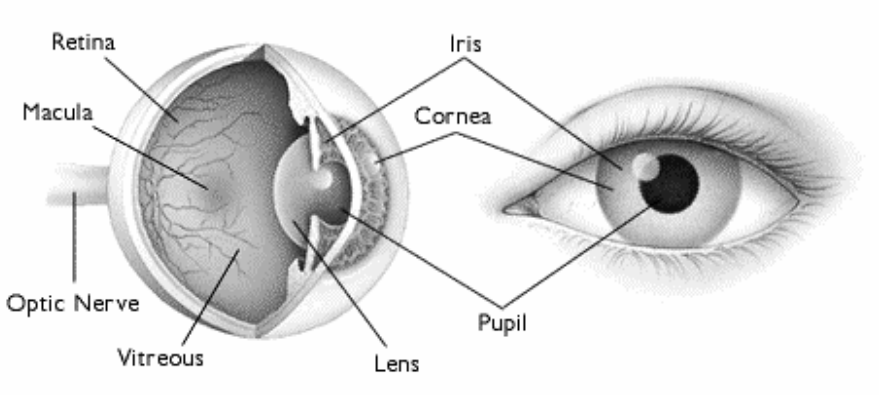


Figure B-1 Anatomical diagram of the eye

(www.castlegate.net/ mebfl/anatomy.htm accessed 10/8/2003)

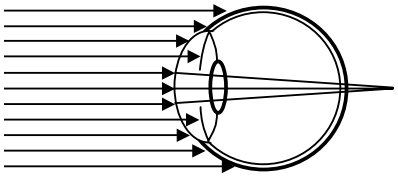
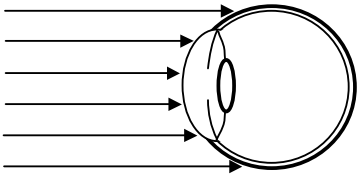
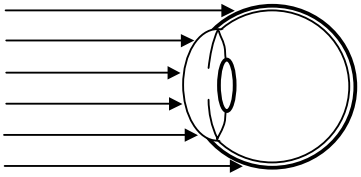
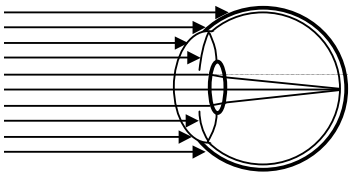
Near Infrared	**Far Infrared**
	
Energy is absorbed in the ocular media and at the retina; near infrared rays are refracted	Absorption is localised at the cornea
Short Ultraviolet	**Long ultraviolet and visible**
	
Below 300 nm, absorption occurs principally at the cornea	Transmission by the cornea gradually increases above 300 nm. Light is absorbed at the retina (some long wavelength UV is absorbed in the lens)

Figure B-2 Damage to eye from laser wavelengths

Retina

The visible and near infrared wavelengths (400-1400nm) can be transmitted through the clear ocular media and be absorbed by the retina. This region of the spectrum is often referred to as the retinal hazard region. The lens of the eye focuses the already highly collimated light into and extremely small spot size on the retina in the order of 10-20μm. This focusing of the beam increases the concentration of the light, after entering the eye, is of the order of 100 000. Thus a beam of $1W/cm^2$ at the cornea will focus on the retinas with an irradiance of $100kW/cm^2$. Whilst this spot size is extremely small its damage can be significant for two reasons. Firstly the spot will fall on the small areas of the retina which are critically important for high acuity vision, the

central retina including the macular its fovea. Secondly the intense radiation will cause significant heat flow and photoacoustic damage to surrounding tissue. This damage is permanent since the neural tissue of the retina has very little ability for repair (Sliney, 1995).

Cornea and Lens

At wavelengths outside this retinal hazard zone injury to the anterior parts of the eye is possible. The cornea is susceptible to damage from a wide variety of wavelengths in the ultraviolet and far infrared band greater than 1400 nm. Normally injury to the cornea is superficial and due to the high metabolic rate it will repair itself in a few days. However if injury occurs in the stroma or endothelium and in the germinative layers of the cornea, corneal scars can occur leading to permanent loss of vision. Generally thresholds for injury to the cornea are much greater then those for the retina. Table B-6 lists lasers, their wavelengths and the part of the eye susceptible to damage.

Table B-6 Laser wavelength and eye damage

Laser	**Colour**		**Wave-length (nm)**	**Susceptible to damage**	**Eye Effect**
Excimer:	Ultraviolet				
ArF	UV-C		193	Cornea	Photokeratitis
KrCl	UV-C		222	Cornea	Photokeratitis
KrF	UV-C		248	Cornea	Photokeratitis
XeCl	UV-B		308	Cornea	Photokeratitis
XeF	UV-A		351	Lens, Cornea	Photochemical, UV Cataract
Argon	Blue	Visible spectrum	488	Retina	Photochemical, thermal retinal injury
	Green		515	Retina	
Krypton	Green		531	Retina	
	Yellow		568	Retina	
	Red		647	Retina	
Dye laser	Variable with dyes Red		632	Retina	
	Yellow/green		577	Retina	
Gold vapour	Red		628	Retina	
Helium neon	Red		632	Retina	
Ruby	Deep Red		694	Retina	
Diode laser	Variable (Red to Near Infrared, IR-A)		670-900	Iris, Retina	Cataract, Retinal burns
Alexandrite	Near Infrared (IR-A)		720-800	Iris, Retina	
Nd:YAG	Near Infrared (IR-A)		1064	Iris, Retina	
			1318	Iris, Retina	
Er:YAG	Near Infrared (IR-B)		2940	Cornea, Retina	Corneal burn, Aqueous flare, IR Cataract
Ho:YAG	Near Infrared (IR-B)		2100	Cornea, Retina	
Carbon dioxide	Far Infrared (IR-C)		10600	Cornea	Corneal burn

Skin

Although the chance of skin exposure to direct or indirect laser beam is much greater the skin is much less vulnerable to injury than the eye. Damage to the skin can occur from either photochemical (produced by ultraviolet wavelengths) or photothermal (visible and infrared) mechanisms depending on the wavelength of the laser. (Sliney, 1995)

The severity of the injury depends upon the length of exposure, the penetration depth of the wavelength, the mode and power settings and whether the contact has occurred via a focused or reflected beam (`specular, diffuse or fresnel reflection`).

Actual thresholds of injury to the skin are normally in the order of joules per square centimeter and this level of exposure does not usually occur outside the focal zone of a surgical laser. (Baxter, 1999) However, with the more widespread use of lasers emitting in the ultraviolet spectral region as well as higher power lasers, skin effects have assumed greater importance (Neimz, 1996 p245). A summary of possible skin damage and the corresponding wavelength is listed in Table B-7 on page 160.

Table B-7 Summary of possible skin damage by laser radiation

Wavelength	**Skin effects**
Ultraviolet C (200-280 nm)	Erythema (sunburn) Skin cancer
Ultraviolet B (280-315 nm)	Accelerated skin aging Increased pigmentation
Ultraviolet A (315-400 nm)	Pigment darkening Skin burn
Visible (400-780 nm)	Photosensitive reactions Skin burn
Infrared A (780-1400 nm)	Skin burn Excessive dry skin
Infrared B (1400nm-3.00 μm)	Skin burn
Infrared C (3.00-1000 μm)	Skin burn

Smoke Plume

As the use of laser to perform surgery has gained wide acceptance in recent years researchers have suggested that the smoke from the vaporisation of tissue and fluid may act as a vector for cancerous cells which may be inhaled by the surgical team and other exposed individuals.

There have been many studies on this subject. One study by Nezhat et al (1987) examined the compositions of the smoke plume produced during carbon dioxide laser surgery to determine whether the operating room team was at risk from the laser smoke. The authors were interested in calculating the probability that something the size of a whole red blood cell (7.5 um) would be present in the smoke. Particles with an aerodynamic diameter range from 0.1-0.8 um were found in the collected smoke plume samples but no cell-size particles, including cancer cells, were present in the plume (probability 0.000001).

The findings of this study differ from some earlier studies in which intact cells or identifiable cell parts were collected from both carbon dioxide (CO_2) and neodymium: yttrium-aluminium-garnet (Nd:YAG) laser radiation of animal tissue. Nezhat et al (1987) concluded that although no identifiable hazard from airborne cancer cells was detected, a significant portion of the particles in the smoke was in the range of 0.5-5.0μm. These particles are too small to be effectively filtered by surgical masks. It was recommended that a mechanical smoke evacuator system with a high-efficiency multi-stage filter be used during smoke generating laser vaporisation procedures.

Fisher (1987) indicated that although mechanical smoke vacuuming systems were used in carbon dioxide laser surgery, the tube had to be held as close as 1 cm from the target. At 2 cm, the evacuation ratio was down to 50%. The author concluded that prudence should be exercised while the hazards presented by the laser smoke are further investigated.

Garden et al (1988) analysed the vapour produced by the carbon dioxide laser during vaporisation of papillomavirus infected verrucae. This study concluded that intact viral deoxyribonucleic acid (DNA) was liberated into the air with the plume of laser-treated verrucae. Papillomavirus DNA has been demonstrated to be infectious. Therefore, when performing laser therapy on patients infected with viruses such as hepatitis or the human immunodeficiency virus, the smoke plume should be assumed to be infectious and appropriate precautions, such as a well maintained vacuum apparatus should be observed.

Appendix C Types of Lasers used in Medicine

Gas

The gas which acts as the lasing medium is contained in a tube of glass or metal with mirrors at either end, creating the optical cavity. Gas lasers typically employ an electrical current as the pumping method. The current is applied to either end of the tube, and is arced through the tube. The flowing electrons of the current excite the atoms they hit as they travel through the tube.

Gas lasers can be very high power, into the kilowatt range and in this power range the applications fall into the material processing area i.e. cutting, marking, welding, heating etc. However there are lower power versions which have found applications in surgery and medicine.

Helium-neon

Javan et al (1960) built the first gas laser utilising Helium-Neon (He-Ne) as the lasing medium (Beesley, 1992 p17). The laser works by passing an electrical discharge along a gas filled tube, much in the same way as with neon signs. The electrical current ionises the helium so that when these collide with neon atoms they become excited as well and provide the condition for stimulated emission to occur. A Helium-Neon laser is a four level laser and therefore efficient and cheap to make and hence it popularity. The Helium-neon laser has a wavelength of 633 nm and a CW typical operational mode.

Initially low power Helium-Neon lasers were considered to have no biological effects and were incorporated into high power lasers which operated in the invisible part of the spectrum as pointers for targeting the main laser beam.

Later research reported Helium-Neon and other low power red or near infrared light sources have a *biostimulation* effect - a photochemical response to laser light inducing a biochemical alterations in cells, leading to physiological changes. They are promoted for wound healing and for pain relief in conditions such as rheumatoid arthritis and osteoarthritis. However most claims of benefits from laser therapy have been exaggerated, especially by the manufacturers of the laser systems leading to scepticism in some area of the medical and paramedical profession.

Argon

Unlike the Helium-Neon laser (see page 162) the argon laser works on a transition between two energy levels of the ionised atom. In order to singly ionise argon atoms ie to remove one electron from each atom, a considerable amount of energy must be supplied to the argon gas. In consequence the power supplies for an argon laser are bulkier and more complex than for the helium-neon laser although the power outputs available are very much higher. However, the overall efficiency is about the same (Beesley, 1992 p110).

The argon laser produces an intense visible blue - green light at a number of wavelengths but 80% of the energy is at wavelengths of 488nm and 515nm, (blue and green respectively). In clinical use, the combination of blue and green light allows for more complete tissue absorption and in areas where absorption is critical e.g. ophthalmology a green only argon laser is available.

Argon laser energy is transmitted through clear fluids and structures but is readily absorbed by darker tissue which contains haemoglobin, melanin and other similar pigmentation. Because of this selective absorption, the localised heat generation controls the spread of the thermal energy and decreases laser damage to adjacent tissue. Due to its ability to transmit readily through clear fluids, the argon laser was first used in ophthalmology to treat diabetic retinopathy through, and without damage to, the clear anterior parts of the eye (Taka el at 1998).

Whilst the argon laser has found its major application in ophthalmology it has also been used, either experimentally or for clinical procedures in, dermatology, plastic surgery, gastroenterology, gynaecology, forensic science and tissue welding as summarised in Table C-1on page 165.

Argon Laser Delivery Systems

Argon laser energy can be delivered to the tissue through a slit lamp, microscope or fiber, controlled by a foot switch or hand control button.

The fiber delivery method is usually used in the noncontact method and as the beam diverges approximately 10 to 14^0 at the fiber end changing the distance of the fiber tip from the tissue can alter the spot size.

Handpieces of various shapes can also be fitted to the fiber to deliver the energy to the tissue. There are two basic types of handpieces: a focused device ensures that the spot size is a specific diameter and a hand piece that contains an internal lens that can be moved to change the focal length and spot size of the beam (Ball, 1990).

Table C-1 Use of argon lasers in medicine (including experimental work)

Procedure	**Author, year**
Forensic Science	Shipp et al, 1993; Platt, 1982
Dermatology	Apfelberg et al, 1984; Henderson et al, 1984; Hulsbergen-Henning et al, 1986; Sunde et al, 1990;
Dentistry	Kelsey et al, 1991
Opthalmology	Migdal and Hitchings R, 1985;
Urology	Frank F et al, 1982
Cardiology	Choy et al, 1984; Ginsburg et al, 1984; Cote et al, 1989; Saksena and Gielchinsky, 1990
Neurology	Davaux and Roux, 1996
Tissue welding	Kilkelly et al, 1996

Carbon Dioxide (CO_2)

The active medium in the carbon dioxide laser is really a combination of specific concentrations of carbon dioxide, nitrogen (or argon), and helium gases in the ration of 1:1.5:4. CO_2 is the active medium and the addition of the two other gases are designed to increase the efficiency of the output. Exciting this active medium with an electrical current produces a beam at 10.6μm wavelength, in the far infrared spectrum.

In the conventional design of *Lasers Surg Med* lasers, a gas mixture flows through a hollow laser cavity where the CO_2 gas mixture is energised by high voltage electrical discharge. This stimulates emission of coherent light, but it results in disassociation of the active CO_2 molecule into carbon monoxide and a free oxygen radical. This fractured CO_2 molecule is no longer able to produce coherent light and must be either pumped away or recombined. Lasers that replenish

used CO_2 with fresh CO_2 molecules are called 'flowing gas systems'. Historically, they carried the advantages of reliable, steady output and easy generation of high powers. However, gas pumps make the laser bulky and noisy and add substantially to operating costs. A sealed tube laser eliminates the need for replacement of the gas mixture. In the past, sealed tube lasers were excited by direct current. This is an inefficient mechanism, which often sacrificed operating power. The latest excitation mechanism uses radio frequency current to produce a laser capable of sustaining average powers in superpulsed mode that had been previously unobtainable (Reid and Absten, 1994).

The CO_2 beam is strongly absorbed by water in tissue. Since biological tissue contains 75% to 90% water, the beam is readily absorbed and heats the cellular contents. Ninety-seven percent (97%) of its energy is absorbed at its impact site on skin and 98% is absorbed in water at a depth of <0.5mm. Thus, even the 3 % of energy reflected in skin is absorbed totally after passing through a very short distance in adjacent tissue. Adjacent tissue is affected only by thermal transmission from the target cells and the end result is a relatively small amount of thermal damage to tissue not in direct contact with the carbon dioxide beam. In skin, the thermal damage extends for only 30 to 50 μm from the impact site. The impact site on the other hand, receives virtually the total power output of the CO_2 laser, resulting in the instantaneous transformation of tissue water to steam and, thus vaporisation or evaporation (Bailin et al, 1990 p141).

The power from the CO_2 laser may be concentrated or diluted, determined by the desired clinical end-point. Concentration of laser energy results in its ability to excise (cut). Dilution of its energy, on the other hand, causes tissue vaporization. Thus the CO_2 laser is best known for its precision and cutting properties. It is often used as a laser scalpel.

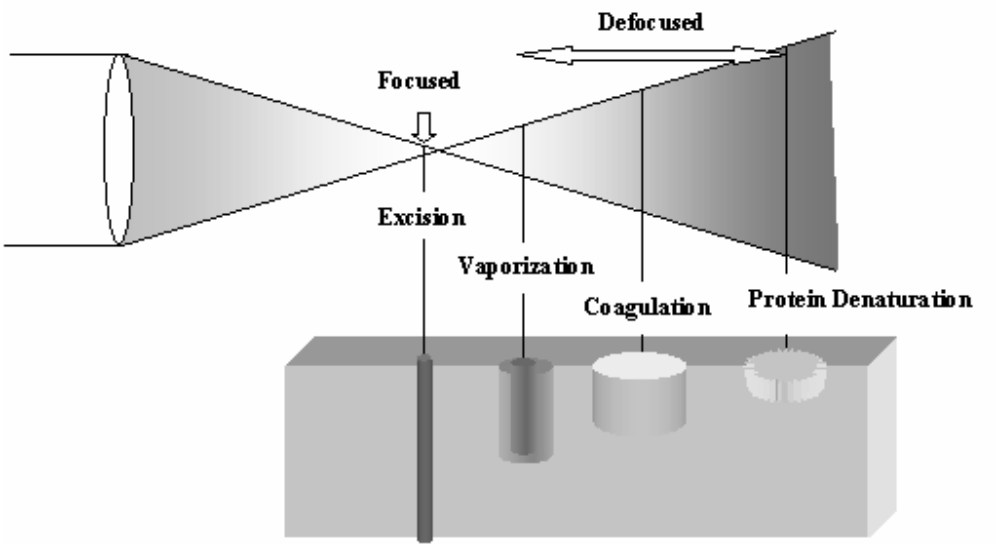


Figure C-1 Spot size and tissue effect

(Bailin et al, 1990, p143)

Since the beam is invisible, a helium-neon laserlight is transmitted coaxially with the CO_2 laser light and serves as the aiming beam. Transmission of the CO_2 beam is through articulating arms with mirrors.

Unlike the argon laser the absorption of the CO_2 is independent of the tissue colour. Therefore lighter tissue can absorb the beam in the same proportion as darker tissue. The CO_2 laser also has less scattering than the Nd:YAG laser.

The lateral zone of damage or the depth of penetration of the CO_2 laser beam is at maximum 0.5 mm and the power density and the duration of exposure can control this depth of penetration.

Depending on the type of treatment, CO_2 lasers can be operated in three different modes CW, chopped pulse and superpulse. Refer to Figure C-2 below.

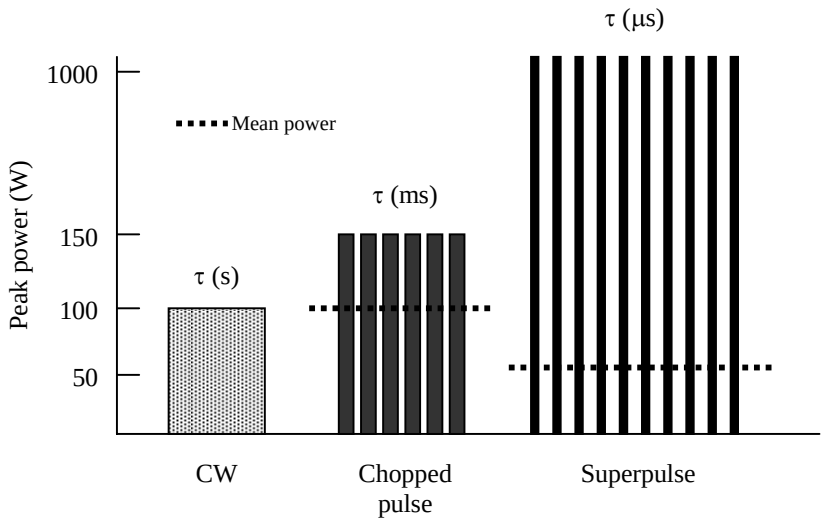


Figure C-2 Modes of operation of CO_2 laser

Several major advantages of the CO_2 laser include its ability to seal vessels and nerves and to minimise the amount of scarring while enhancing the positive qualities of the resulting scar. Its ability to seal blood vessels of 0.5 mm or greater results in hemostasis, and similar sealing of lymphatics may have important ramifications during tumor surgery. The sealing or capping of cut nerve endings results in a marked diminution or total absence of pain postoperatively, and the effect of infrared radiation on fibroblasts may be responsible for minimizing postoperative scarring (Bailin et al, 1990, p145).

Probably the most singular advantage of the CO_2 laser is its ability to vaporise tissue. This is usually accomplished with in the impact spot defocused to a size of 2 to 3 mm. The depth of the vaporisation can be determined, to some extent, by the power density or irradiance of the CO_2 laser beam at tissue. Such vaporisation can take place slowly and purposefully by deliver laser pulses in chopped mode or can be done more rapidly through the delivery in superpulsed mode.

The most rapid vaporisation can occur with the laser operating in the continuous mode (Bailin et al, 1990 p 147)

Metal vapour

An important group of lasers are those having a metal vapour excited in a discharge with a noble gas as the buffer. Many of the metallic elements in the periodic table can be made to lase in this way. The Helium-Cadimum laser has attracted the most commercial interest due to its ability to produce CW emission at 441.6nm and 325nm, however these frequencies have yet to be utilised in medicine.

In metal vapour lasers, the solid metal is first vaporised and then brought into the discharge tube to be ionized. Eventually all the metal is transported to one end of the tube (the anode). Thus when all the metal has been vaporised the laser ceases to operate. To avoid this, either a return path is provided to recover the metal or a cathode at the opposite end of the tube is alternated with the anode.

The two most common metal vapour lasers found in medicine and in particular dermatology are the copper vapour and gold vapour lasers.

Excimer

Gas lasers use an inert gas whose atoms gain and lose energy, but remain elementally intact however, the excimer laser incorporates multiple types of gasses that react and form new molecules when excited, and then decay back into their original constituents. The active medium is a combination of a rare gas with a halide, usually a halide-oxide, or a halide-halide dimer. The dimeric mediums are excited to emit laser energy. There are approximately twelve lasers in this class of which only three or four are in present use in medicine or medical research.

Depending upon the exact chemical composition of the active medium, a variety of ultraviolet wavelengths can be produced. UV radiation is extremely strongly absorbed by most biological molecules in a wavelength band between 200 and 300 nm. This feature has recently been exploited to produce well defined, non-necrotic photoablative cuts of very small widths (~ 50 micrometers) by exposure to excimer laser wavelengths with short pulses (~10ns) focused on tissue (~10e8 W/cm^2) (www.rli.com/excimer1.html)

The four most popular gases used in the Excimer laser range are argon fluoride (ArF), producing a wavelength of 193 nm, krypton fluoride (KrF), 248 nm, xenon chloride (XeCl), 308 nm, and xenon fluoride (XeF), 351 nm. The hazard associated with the excimer laser system is that most of the gases used in the unit are extremely toxic and even fatal. The challenge has been to develop a laser system designed to encapsulate these toxic gases and to provide protection from high - voltage discharges.

Many publications refer to the excimer laser as producing a *cool* beam of light despite the fact that it does produce heat in the target material. The beam was thought to interact with biological tissue through a photochemical process whereby the light breaks the molecular bonds between cells and vaporises tissue, one microscopic layer at a time. Thus excimer lasers are often thought of as nonthermal lasers. This was substantially different from most surgical laser systems which effect tissue by producing heat that burns it, or by producing a shock wave that separates tissue with micro-explosions. However the excimer ablation is a thermal process (Neev et al, 1993) and the *clean* tissue effects which are ascribed to the wavelength of the excimer laser are usually due to the natural pulsing of this class of laser (Absten and Joffe, 1993 p34).

The excimer laser has the ability to precisely sculpt with almost no effect on the surrounding tissue. This ability makes it ideal in ophthalmic work, in particular shaping of the cornea. (http://www.rli.com/excimer1.html) It has also found uses in dentistry and for bone ablation. (Izatt et al, 1990; Izatt et al, 1991; Arima and Matsumoto, 1993)

Liquid

Some liquids which are usually organic dyes can be made to lase. The use of liquids as the active medium of a laser is obviously attractive as liquids can be of very high optical quality and the cooling problems which often arise in solid-state media can be overcome by circulating the liquid. Furthermore it has been found that liquid lasers can be tuned easily by a number of different methods over a wide range of frequencies. High quality solid state laser crystals are usually extremely expensive whereas the cost of suitable liquids is almost negligible.

Dye lasers

Dye lasers incorporate an organic dye dissolved in an alcoholic solvent of a specific molar concentration. This liquid solution is exposed to an intense light source, usually an argon laser beam. The dye absorbs the laser light and then fluoresces over a broad spectrum of colours. These are remarkable because they are tunable, that is the wavelength of the emitted light can be changed by adjusting certain operation parameters of the laser unit, usually the direfringent crystal and can be tuned from 400 to 1000nm. They can be used in either the continuous or pulsed modes. These varying parameters increase the lasers applications.

Tuning the dye laser to 577 nm causes highly selective vascular damage in clinical applications. Since haemoglobin has a local absorption peak of 577 nm, a vascular lesion will readily absorb this laser energy, while the nonvascularized tissue will cause the beam to diffuse. Thus, the

lightening of a portwine stain can by successfully achieved by using this wavelength (Ball 1992) whilst a wavelength of 630nm is used in photodynamic therapy. They are also used for spectroscopy, cell sorting, laboratory counting devices, dermatology, and selective destruction of malignancies. Table C-2 shows various wavelengths of the Tunable Dye lasers and some medical applications.

However it has been noted that the medical applications of dye lasers will be rather limited because of the inconvenience and complicated maintenance. In contrast to the long living solid-state crystals, dyes need to be recirculated and exchanged on a regular basis which often disables a 'push button' operation.

Table C-2 Various types of medical dye lasers and uses

Laser wavelength (nanometers)	**Medical usage**
577-585 nm (yellow light)	Cosmetic surgery in particular vascular lesions
504 nm (pulsed)	Fragmentation of kidney stones and gallstones
630nm (red light; CW)	Photodynamic therapy

Solid state

Solid state lasers use a crystal of some material which has been doped with an impurity. The laser relies on the atoms of the impurity to become excited, usually employing optical pumping from high energy xenon flash lamps or from a second pump laser or laser diode array and then decay to form the coherent photon beam.

Ruby

The first laser to be constructed by Maiman in 1960 used ruby as the active medium. Ruby is the common name for a sapphire host crystal doped with small amounts of Chromium ions, Cr^{3+} where electrons are excited between three energy levels and can produce light which has a wavelength of 694.3nm. A ruby laser when it is Q-Switched still provides a most powerful and useful source of light. The ruby laser was largely overlooked in medicine due to its unsuitability for many of the earlier medical applications which were investigated by laser researchers however it resurfaced in the 1980's for the treatment of tattoos and benign pigmented skin lesions.

Nd:YAG (Neodymium: Yttrium Aluminium Garnet)

The Nd:YAG laser consists of a solid crystal of yttrium aluminium garnet. It is doped with a rare earth, neodymium that actually produces the laser light energy when exposed to bright flash lamps. There are three frequencies which can be produced by this crystal. The Nd:YAG laser is located in the near infrared region of the electromagnetic spectrum with a usual wavelength of 1064nm. This can be modified to produce the frequency double Nd:YAG with a wavelength of 532 nm (see KTP below). The latest Nd:YAG laser to be introduced produces a wavelength of 1318nm.

The Nd:YAG laser can be operated in the continuous CW mode or in a pulsed mode. The CW laser system has special power requirements and some systems require external plumbing connections to provide a constant flow of cool water. Other systems are air cooled with an internal closed water supply cooled by a fan.

KTP

The potassium titanyl phosphate (KTP or KYP/532) laser lases at 532 nm in a quasi-CW mode. The lasing source is a Nd:YAG laser in which the laser rod is continuously pumped with a krypton arc lamp and Q-switched. The 1.06um light traverses a frequency doubling potassium titanyl phosphate crystal yielding the 532 nm green light. The laser normally does not have any special power requirements; does not require external cooling water and can easily generate up to 20 Watts of power. This method of beam production is substantially more efficient than the argon ion laser (Reid and Absten, 1995).

Similar in frequency to the argon laser the KTP laser has similar characteristics. It is readily transmitted though clear aqueous tissue because it has a low water absorption coefficient. The wavelength is centred on the haemoglobin absorption band and this and other similar tissue pigments such a melanin absorb the KTP laser light effectively. These absorption characteristics combined with the effect of interpulse cooling reduces secondary heat conduction and hence makes it useful for dermatology and ophthmalogy.

Er:YAG (Erbium: Yttrium Aluminum Garnet)

The Er:YAG laser emits high energy pulses of mid-infrared light with a wavelength of 2940 nm (2.9um). This wavelength corresponds to maximum absorption by water, therefore penetration depth in the tissue is minimal and hence thermal relaxation time is short. If this is combined with a short pulse width it can provide precise control with an exceptionally narrow zone of damage around the vaporisation crater. Hence the Er:YAG has been tried for corneal resulting, a task generally reserved for the excimer laser. The Er:YAG laser is also very highly absorbed by osseous minerals, making it a highly precise drill and saw for bone surgery and dentistry (Reid and Absten, 1995).

Ho:YAG (Holmium: Yttrium Aluminum Garnet)

The Holmium: Yttrium Aluminium Garnet laser is the newest laser in the YAG range. It emits a wavelength of in the mid-infrared range of 2100nm. The active medium is Holmium, a rare earth element, within a yttrium aluminium garnet crystal. The active medium is excited by a Xenon arc flashlamp and currently produces up to 100 watts of laser power, usually at 0.25 millisecond pulses.

Like the other mid-infrared range lasers (CO_2 and Er:YAG) the laser energy is strongly absorbed by water, making it useful fool for ablating most soft tissues. However unlike the CO_2 laser Ho:YAG energy can be delivered through a flexible optical glass fibre and can be operated effectively in an aqueous environment (Trost et al, 1992; Reid and Absten, 1995).

Although the Ho:YAG energy has a slightly linger absorption length than CO_2 energy, the strongly pulsed nature of the laser emission and the freedom from "thermal blooming" allow the laser to make a finer incision than an endoscopically delivered CO_2 beam (Reid and Absten, 1995).

Semiconductor

Semiconductor lasers are built around layers of silicon in the form of a semiconductor diode. The lasing medium is the silicon material. Semiconductor lasers use an electrical flow across the diode as the pumping method. The optical cavity is usually a small metal can in which the diode is encapsulated. Semiconductor lasers are very small as compared to the other forms of laser.

Semiconductor lasers create beams in several wavelengths, ranging from the visible spectrum to near infrared.

Appendix D Applications of Lasers in medicine

The future applicability of lasers in medicine was evident when the first laser was developed some 40 years ago. In fact laser surgery equipment was in the market place in 1965, less than 5 years after the first working laser was developed (Ossoff, 1994). Since then, laser based techniques have been in continuous development in medial research, diagnostics and therapeutics. Each different type of laser operates on a similar principal but because of the different wavelengths of light emitted, their applications differ. However all of the utilise thermal energy generated by light and tissue interactions.

Lasers were firstly used in ophthalmology and dentistry then in ear, nose and throat surgery, gynaecology and neurosurgery. Development spans from the ruby and argon lasers in outpatient eye surgery to the CO_2 laser and Nd:YAG lasers in the operating theatres and further on to the PDT- dye lasers, excimer and solid state angioplasty laser.

This wide ranging treatment of many disorders in many medical specialties and surgery can be primarily attributed to the rapid development of pulsed laser systems as it is the pulse duration which finally determines the effect on biological tissue. Many treatments are still in an experimental stage, but in many areas the laser treatment has become fully accepted. In a few cases e.g. ophthalmology the laser is the only instrument available and has supplanted traditional surgical approach. The laser can be used to cut, as in surgery, to coagulate as used in dermatology, to destroy as in cancer treatment, and to stimulate as in pain relief. Choosing the best laser for a given surgical application depends on four things (1) the absorption characteristics of the tissue; (2) the wavelength of the emitted radiation; (3) temporal parameters of the

delivered energy and (4) mechanisms of beam delivery (Reid and Absten, 1995). An overview of major areas of medicine and the corresponding laser usage is given in Table D-1.

Table D-1 Laser usage in major area of medicine

Application	**Lasers used**
Dermatology	Argon, CO_2, Nd:YAG, ruby, metal vapour, pulsed dye, gold vapour
Plastic Surgery	Argon, CO_2, Nd:YAG, ruby
ENT	CO_2, Nd:YAG
Gastroenterology	Argon, Nd:YAG
Gynecology	Argon, CO_2, Nd:YAG, Ho:YAG, KTP
Oral Surgery	CO_2, Nd:YAG
Ophthalmology	Argon krypton, pulsed Nd:YAG, excimer, dye, diode
Photodynamic therapy (PDT)	Gold vapour, argon pumped dye, copper vapour pumped dye
Pulmonology	Nd:YAG
Urology	Nd:YAG
Angioplasty	Argon, Nd:YAG, excimer, pulsed dye, Ho:YAG
Tissue welding	Argon, CO_2, Nd:YAG, diode
Neurosurgery	CO_2, Nd:YAG
General surgery	CO_2, Nd:YAG
Stone fragmentation	Pulsed dye, pulsed Nd:YAG, excimer, Ho:YAG, alexandrite
Orthopedics	Ho:YAG

The potential advantages of various lasers include: reduced blood loss; dry surgical field; reduced edema; limited fibrosis and stenosis; fiberoptic delivery capabilities; no interference with monitoring equipment; potential reduction in spread of metastases; precision; fewer instruments in the operating field; reduced postoperative pain; sterilization of the treatment site; contact or no-touch techniques.

The main disadvantage to lasers in the medical field appears to be that like any other technique or tool, the success or otherwise, is largely dependent on the skill of the surgeon or operator. However unlike accepted surgical procedures the acquiring of this skill is largely left to the surgeon and falls largely under the heading of *continuing professional development.* Unless the technique has totally replaced normal surgical procedures as in the case of ophthalmology, the laser technique is not taught as part of the normal training.

General surgery

Lasers used as a surgical knife has shown certain advantages over scalpels, electrocautery and cryosurgery, as the laser surgery is a non contact method, bloodless, precise with better visualization, minimum postoperative edema, painless healing without complications (Taka et al 1998 abstract only).

The development of "contact probes", in particular for the Nd:YAG laser by Joffe (1984) has been invaluable in the progress of lasers in general surgery. The restoration of tactile feedback to the surgeon has been the greatest advantage of the contact probes. This type of laser delivery system has been used to provide a hemostatic effect with partial resection of vascular organs, such as the liver, spleen, and pancreas. In addition its endoscopic application in the hepatobiliary tract may prove helpful in the treatment of obstructing carcinomas of the bile duct (Shapshay, 1988).

Dermatology

Dermatology includes problems of the skin, hair, mucous membranes and nails. These problems range from prevention and treatment of cosmetic problems, such as undesired tattoos, leg veins and acne scars to severe skin ulcers and skin cancers. The range of therapeutic methods used in dermatology is also wide, including microscopically controlled cancer surgery (Mohs), laser

therapy, phototherapy and freezing (cryotherapy). (http://www.med.miami.edu/dermatology/dermintr.htm as at 18/4/00)

Dr Leon Goldman pioneered the use of the laser in dermatologic surgical procedures in 1963 (Roenigk, 1994; Goldman L, 1978 p861) and other early laser studies noting and assessing tissue reaction to laser energy have led to significant advances and clinical applications in dermatology, in some cases long before other areas of medicine. They have been used in the treatment of skin conditions for more than 30 years and the application of laser technology to dermatological conditions in Australia started approximately 27 years ago with the use of the CO_2 laser for the ablation of tattoos (Stewart et al, 1994). Overall, the effectiveness of laser treatment has been established in a number of dermatological applications and this is probably the fastest growing area of medical laser use in Australia (Dankiw et al, 1993).

Lasers are versatile instruments capable of treating an ever increasing array of skin conditions. Various vascular, pigmented, epidermal and dermal lesions can be selectively destroyed by using lasers that correspond to the absorption characteristics of the intended target without damaging normal healthy surrounding skin structure such as epidermal pigment and dermal collagen. Skin treated by this method normalizes in colour, texture and its markings (Rosenbach and Alster, 1996; Tan and Morelli, 1991).

The CO_2 laser has evolved and is now one of the more versatile laser systems in dermatology and has many clinical applications. Many skin disorders limited to the epidermis or superficial dermis can be effectively vaporised using the CO_2 laser (Roenigk, 1994). It has been used in the treatment of verruca vulgaris (warts) (Mcburney and Rosen, 1984) actinic cheilitis (sun induced keratoses of the lip) (David, 1985), keloids (Kantor, 1985), condyloma acuminata (gential warts) (Baggish, 1985) and cutaneous malignancy (Waters, 1991). Found in clinical use are also many

other laser systems including the argon, Nd:YAG, ruby, metal vapour and dye lasers although some of these systems have a very specific and limited application.

However the rapid development of laser technology has made many lasers obsolete that only a few years ago were considered state of the art. Even leading publications are quickly dated e.g. in an article by Bailin et al (1990, p161) quoted "As for yellow light therapy, no such potential is thought to exist for therapy of vascular lesions." Just 4 years later the Australian Institute of Health and Welfare, Canberra produced a government publication titled "Yellow light lasers in dermatology: Laser treatment of superficial cutaneous vascular lesions". This publication went on to summarise the use of yellow light lasers in the treatment of such dermatological conditions as port wine stains, telangiectasia, spider naevi and rosacea cases.

The discussion of lasers in dermatology has been covered by addressing at the main areas of dermatology: vascular lesions (portwine stains, pigmented lesions and telangiectasia); cutaneous lesions (skin incisions, scars and keloids, inflammatory lesions and verruca); cosmetic surgery (tattoo removal and skin resurfacing) and laser blepharoplasty.

Vascular lesions

Vascular lesions of the skin include portwine stains, pigmented lesions and telangiectasia and are commonly treated by yellow light since this frequency is absorbed by haemoglobin. This selective absorption results in a much reduced risk or scar formation based on the concepts of selective photothermolysis (Roengik R, 1994). Although with less efficacy than lasers with slightly longer wavelengths, the argon laser is a yellow light laser and hence is absorbed by pigmentation and melanin; this laser is appropriate for use in treating vascular lesions. The argon laser's depth of penetration is approximately 0.5 to 2 mm, vascular lesions located deeper in the dermis do not respond well to argon laser energy. However, vascular lesions that are in the upper

dermal layer respond readily to argon laser treatment (Ball, 1990). The best results for treatment of superficial vascular lesions have been reported with KTP (532nm) and flashlamp excited dye at a wavelength of 585nm (Reongik, 1994 and Rosis, 1993).

The relatively new technique of utilizing photoabalation techniques with the deeper penetration of the Nd:YAG wavelengths has been used successfully in the treatment of cavernous hemangiomas of the skin, bulky portwine stains and lesions associated with Osler-Welber-Rendu disease. The laser is used with low-power settings of 20 to 30 W at intermittent 0.5 to 1.0 second exposures to photoablate these lesions without disrupting the overlying epithelial or skin cover (Shapshay, 1988).

Portwine stains

The ruby laser was first used in an attempt to treat port wine stains (a bluish-red vascular malformation of the skin). However because of its lack of absorption by haemoglobin, damage was caused to the surrounding tissues and this approach was quickly abandoned.

Until the 1980s the argon laser was the treatment of choice for port wine stains (Phillips, 1992). A good response to treatment could be expected in 60-80 % of patients, with the best results observed in those adults with purple port wine stains (Dixon, 1984 ; Stewart et al, 1994).

Complications that may result from argon laser lightening of portwine stains are scarring, hypopigmentation and skin texture changes. Hypertrophic scarring is the most significant complication. It is theorized to be caused by the laser's thermal spread and zone of destruction. Even though this complication is not common, the areas most affected are on the thin tissue of the lateral and anterior neck and the moustache portion of the upper lip (Ball, 1990).

Lanigan et al (1989) reported in the British Journal of Dermatology on the treatment of 100 patients with portwine stains with a rhodamine dye laser emitting at 577 nm. They established that this laser was an effective treatment in about two thirds of patients (Lanigan et al, 1989).

Lanigan and Cotterill (1990) discussed the results of a study aimed to evaluate the effects of the CO_2 laser in the treatment of port wine stains in adults. These patients had failed to respond to either argon or dye laser treatment or were children with pink and easily compressible port wine stains which would not be expected to respond well to other lasers.

Seventy four percent of the adults and 53 percent of the children obtained an excellent or good result. However the general opinion does not favour the use of the CO_2 laser, particularly in children because of the potential for scarring, however it can provide significant lightening by vaporising the surface epithelium and sealing the superficial dermal plexus of the distended small blood vessels. The preferred laser for treatment of children, especially under the age of 5 years, is the flashlamp excited dye laser. This laser provides a high peak power in a short pulse thereby fulfilling the criteria of selective photothermolysis (Roenigk, 1994).

Pigmented lesions

A hemangioma is a benign tumour of dilated blood vessels. Since the argon laser is selectively absorbed by haemoglobin from the blood in the vessels, this wavelength can very effectively lighten these lesions (Ball, 1990).

Ashinoff and Geronemus (1991) treated 10 children with strawberry hemangiomas with flash lamp pumped pulsed dye laser and reported the results. All patients responded well to the treatment with no ill effects such as ulceration, haemorrhage, infection or scarring and in addition there was no evidence of either hyper of hypopigmentation.

Deep cavernous hemangiomas can be resected with the CO_2 laser because the argon laser energy cannot penetrate the lesion to its fullest. The procedure is relatively bloodless unless large vessels are encountered.

A nevus is a congenital discolouration of the sin caused by pigmentation. These lesions may be removed with the CO_2 or argon lasers. The pigment or melanin that is within the dermis of the skin absorbs the argon wavelength. There is selective destruction of the melanocytes followed by a lightening of the area (Ball, 1990).

Telangiectasia

Telangiectasia is caused by the dilatation of capillaries and sometimes terminal arteries. This lesion may appear as a birthmark and is most frequently found on the face and thighs (Ball 1990).

Increased powers of argon energy are needed to achieve blanching of the telangiectasia. Power of from one to three watts is used in short pulse durations of between 0.05 and 1 second. A series of laser beam impacts are directed along the vessels to produce tiny weld spots. This in turn stops the blood flow and leads to a blanching effect.

Dilated or branched capillaries on the skin cause spider angiomas or superficial varicosities. Lesions of this type have been successfully treated on the face and neck, but varicosities on the legs have not responded well to treatment (Ball 1990).

Many techniques have been investigated over the years to treat spider veins of the legs. The argon, CO_2 contact YAG, green only argon and frequency doubled YAG system have been used with moderate success (Ball 1990).

Apfelberg (1987) has reported success with yellow light laser energy at 577 nm being delivered to the tissue using high-powered magnification.

In the treatment of vascular lesions, the target chromophore is oxyhaimoglobin. Yellow light lasers with wavelengths in the vicinity of 577-585 nm target this chromophore with less uptake of the laser energy by melanin, the competing chromophore. Thermal damage is limited by selecting a pulse duration shorter than the thermal relaxation time of the target tissue (Anderson and Parrish, 1983).

Wavelengths longer than 585 nm should theoretically provide for even deeper penetration (Tan et al 1989). The Long-Pulse Dye laser at 595 nm was used to treat leg telangiectasis by Hsia et al (1997) with results of greater than 50 % clearance in 64.7% of patients (clearance by 5 months after treatment using 18J/cm2).

Cutaneous lesions

Scars and keloids

Initially various CW lasers such as argon, Nd:YAG, CO_2 were used to treat hypertrophic scars and keloids, however recurrences with in two years were uniformly observed and benefits not clearly established (Norris, 1991; Apfelberg et al, 1989; Hulsbergen-Henning et al, 1986; Kantor et al, 1985; Henderson et al, 1984; Apfelberg et al, 1984).

In the last 10 years the pulsed dye laser (585 nm) has been shown in several studies to successfully treat hypertrophic scars and keloids. (Alster et al, 1993; Alster 1994; DierickxC et al, 1995; Alster and Williams, 1995; Alster, 1996; Alster, 1997)

Cosmetic surgery and Plastic surgery

Tattoo removal

A tattoo is made by injecting pigmentation into the skin. Commercially made tattoos are more uniform in depth of penetration, as contrasted to homemade tattoos, which have an irregular depth of penetration. However professional tattoos are more difficult to treat as they are comprised of multicoloured organometallic dyes and are placed deeper and are more densely packed in the skin.

Argon, CO_2 and Nd:YAG lasers have shown to be effective in removing tattoos as reported by Apfleberg et al (1979) and Reid and Muller (1980). However older laser systems such as the CW CO_2 and argon lasers also led to scarring due to excessive thermal injury of normal skin and thus the use of the CO_2 laser this is now not the preferred treatment for tattoo removal. (Alster and Bettencourt, 1998) Other studies by Scheibner et al (1990) and Taylor et al (1990) have shown success with the ruby laser, although it does depend of the dyes used in the tattoo.

The 1993 annual meeting of the American Society for Laser Medicine and Surgery (ASLMS, Wausau, WI) reported a growing interest in the use of Q-switched ruby, Nd:YAG and alexandrite lasers for tattoo removal due to the fact that Q-Switched lasers that produce ultra short pulses of high energies that literally shatter tattoo ink particles with out destruction of the surrounding tissue (Alster and Bettencourt, 1998). Researches were finding that each of these wavelengths is appropriate for treating different tattoo inks, and thus a multiwavelength system is needed (Kincade, 1993). This is an advantage as it is extremely important that all dye particles are removed during the same session.

Table D-2 Laser type and effect on tattoo dye

(Alster and Bettencourt, 1998)

	Tattoo Dye			
Laser Type (wavlength nm)	**Black**	**Green**	**Red**	**Tan**
Ruby (694)	+++	++	-	Darkening
Alexandrite (755)	+++	+++	-	Darkening
Nd:YAG (1064)	+++	+	-	Darkening
Frequency doubled Nd:YAG (532)	-	-	+++	Darkening
Flashlamp-pumped pulsed dye (510)	-	-	+++	Darkening

Otorhinolaryngology

The use of lasers in otolaryngology dates to the late 1960's with experimental work by Sataloff, (1967); Hogberg (1967) and Jako (1972) and their colleagues on the middle ear, labyrinth and human vocal folds respectively. However these early attempts were not particularly successful due to the low intensity levels and poor absorption by the tissue. Research continued and today applications of lasers include microlaryngeal surgery, treatment of diseases of the mouth and tongue, otology, nasal and sinus surgery (Absten and Joffe, 1993 p48). The CO_2 laser is the most widely used however several studies indicate success with a wide variety of others (Absten and Joffe, 1993 p48; Shapshay, 1988; Stevens, 1990).

Gibson and Kernohan (1993) in a review of lasers in medicine indicated that the CO_2 laser is widely used in the removal of oral carcinomas and cancer of the larynx and in conjunction with other conventional treatments for advanced tumours. It is also used for removal of benign or precancerous lesions in the oral cavity, recurrent papillomatosis, tonsillectomy and adenoidectomy.

Although the CO_2 laser was one of the first lasers used for tonsillectomy (Martinez and Akin, 1987) a number of practitioners have successfully used the Nd:YAG contact laser for tonsillectomies (Maloney, 1991; Malouf and Harrington, 1995). Malouf and Harrington (1995) used the bipolar contact ND:YAG laser to successfully perform 374 consecutive tonsillectomies.

The advantages of the bipolar system in addition to the existing advantages of the Nd:YAG system of excellent hemostatic qualities, is that the adverse situations ranging from unwanted tissue destruction to the catastrophic event of and airway fire have been overcome. It was concluded that patients can be safely discharged in the early postoperative period and that the patients treated with the laser system reported no difference in sore throat or return to normal diet as compared with the control group. The advantages of laser tonsillectomy, regardless of the type of laser used appear to be less pain, quicker healing, less blood loss, less operative time and same day discharge. The disadvantages appear to be the cost of the laser system, lack of evidence for accelerated healing, the layer of necrotic proteinaceous debris left in the tonsillar fossa leads to a greater chance of secondary infection which in turns generates greater pain after 1 week and the need for antibiotics (Malouf and Harrington, 1995; Maloney, 1991; Stevens, 1990).

The Nd:YAG laser has also been successfully used for treatment of palliation of obstructing tracheobronchial lesions, palliation of obstructing esophageal lesions, treatment of vascular lesions such as laryngeal cavernous hemangiomas, photocoagulation of lymphatic malformations. The advantages of the Nd:YAG laser for these treatments include excellent hemostatic effects and deep penetration; rigid or flexible bronchoscope and contact probes. The major disadvantage is the unpredictable depth of penetration and because of this lack of precision the Nd:YAG laser has not challenged the role of the CO_2 laser in otolaryngology (Shapshay, 1988; Ossoff et al, 1990).

Other lasers used in the fields of otolaryngology are the argon and KTP lasers. The argon laser has been used to perform stapedectomy procedures (because of its ability to be focused to small sot sizes), lysis of middle ear adhesions and spot welding of grafts in tympanoplasty surgery. The KTP laser has been used for treatment of endobronchial lesions in infants and neonates, tonsillectomy, stapedectomy, excision of acoustic neuroma and excision of benign and malignant laryngeal lesions (Ossoff et al, 1990).

Gastroenterology

In gastroenterology the laser was first used in the 1970s to arrest gastrointestinal haemorrhages but is now widely used in numerous other areas (Schroder, 1985; Nishioka, 1995). Due to the advancement of both endoscopic technology and techniques laser treatment can easily be applied to both upper and lower gastrointestinal tract both safely and non-invasive (Niskoika, 1995; Shapshay, 1988). Whilst the Nd:YAG laser is the predominately used the argon, KTP and tunable dye laser (for PDT treatment of cancerous tumours) have also been used (Absten and Joffe, 1993 p55). Table D-3 lists a summary of endoscopic applications of the Nd:YAG laser.

Bleeding from peptic ulcers, arteriovenous malformations, gastric erosions and Olser-Weber related lesions have been treated with argon, contact and non contact Nd:YAG lasers (Swain et al, 1981; Rutgeerts et al, 1984; Swain et al 1986; Joffe and Dwyer, 1988) with the advantage of reduced need for blood transfusions and the avoidance of major resectional surgery (Absten and Joffe, 1993 p 56).

Lasers have also been used for treatment of tumours both with the thermal method and with photodynamic therapy (Nishioka, 1995).

Table D-3 Gastrointestinal endoscopic applications of the Nd:YAG laser

(Joffe and Dwyer, 1988 p 32 Table 5.2)

Laser Tissue Interaction	Endoscopic application
Coagulation	Acute menorrhage Active bleeding Recent bleeding Stigmata of recent hemorrhage (SRH) Potential bleeding Angiodyplasia Varices Hemorrhoids
Vaporisation	Neoplastic disease Palliation Curative Ancillary (e.g. placement of esophageal prosthesis) Benign stricture or web Biliary disease Stricutres Fracturing gallstones
Cutting	Tumor excision (polyps) Sphincteroplasty Stricture Cyst drainage

Gynaecology

Beside ophthalmology, gynaecology is one of the most significant disciplines for laser applications and they have been used in this area since the early 1970's (Dankiw, 1993 p 9; Gibson and Kernohan, 1993). Lasers are used to treat a variety of gynaecological conditions ranging from external genital procedures to laparoscopy and hysteroscopy.

The CO_2 laser was one of the first laser wavelengths used and is still the most widely used laser in this area due to its absorption characteristics. However other systems used include argon, Nd:YAG and KTP depending on the treatment required, instrument availability and the surgeon's preference. Each system offers different advantages based on its absorption characteristics, hence its ability to vaporize, cut or coagulate the target tissue; delivery method e.g. fibre and contact probes and attachments or instruments available e.g. microscope.

New gynaecological applications were introduced, and with instrumentation refinements, the laser advanced laparoscopic surgery to a fine art. The laser was then found to be useful for cutting, coagulating and vaporising during intra-abdominal procedures. The laser was also coupled with the hysteroscope to perform surgery within the uterus. Other clinical applications continue to be developed by innovative gynaecologists.

Ophthalmology

Ophthalmologists are considered the pioneers of laser surgery and were quick to realise and investigate the potential of laser energy. This is probably due to the fact that since the 1950's the xenon arc lamp had been used for photocoagulation of the retina. With the invention of the ruby laser, ophthalmologists quickly investigated its uses. It was found to be of negligible value for direct vascular treatment, but was effective in controlling proliferative diabetic retinopathy (Beetham et al, 1970; Krauss and Puliafito, 1995). The ophthalmic argon laser was introduced by L'Esperance (1968) buts its full potential was not realised until the early 1970's (Krauss and Puliafito, 1995). Since this time many lasers have moved from research and experimental modes into full clinical use, making ophthalmology the first area in which benefits of medical lasers have become widely established. Today treatments of the eye with lasers can be broken into three main areas based on the type of laser tissue interaction: photocoagulation, photodisruption, and photo therapeutic.

A review of applications of ophthalmology undertaken by O'Neill et al (1992) concluded that laser treatments are quick, relatively painless and well tolerated. This is a great advance from Meyer-Schwickerath's work with the xenon arc lamp which required retrobulbar anaesthesia to reduce eye pain and movement.

Photocoagulation

Today various types of laser are being used for either diagnostic or therapeutic purposes and a summary provided by The Royal Australian College of Ophthalmologists has listed the following current laser applications where the photocoagulation effect of lasers has proven effective in treating a wide range of conditions including diabetic retinopathy, retinal vascular disease, intraocular tumours, retinal tears, open angle glaucoma (Dankiw et al, 1993 p23). The commonly used lasers for photocoagulation include argon, krypton, dye and frequency doubled Nd:YAG.

Photodistuption

The targets of all therapeutic laser treatments of the eye can be classified into front and rear segments. The front segments consist of cornea, sclera, trabeculum, and iris. The rear segments consist of lens, vitreous body, and retina.

Retina

There are six major indications for laser treatment of the retina as summarised by Niemz (1996).

- *Retinal holes* - laser surgery welds the retina to the underlying tissue. The attachment of the coagulated tissue is so strong that further tearing is usually suppressed, however if necessary the procedure can be repeated several times.

- *Retinal detachment* - the separation of the retina from the chorioidea in the back of the eye and can often occur when retinal holes are undetected

- *Diabetic retinopathy* - retinopathy is a non-inflammatory eye disorder caused by changes in the retinal blood vessels. Due to lack of oxygen new blood vessels are formed and if these vessels haemorrhage complete blindness can follow. Early laser therapy of diabetic retinopathy relied on direct and intense targeting of neovascular elements, which often only made the problem worse (L'Esperance FA, 1968 and Krauss and Puliafito, 1995).

  Laser treatment is to place between 1000 and 3000 spots on the peripheral of the retina. This is best done with argon blue-green laser or the krypton red lasers. Peripheral vision is somewhat damaged but the process prevents further deterioration and preserves central vision. (Krauss and Puliafito, 1995)

- *Central vein occlusion* - (blockage of the vein) as a consequence retinal veins become dilated and severe edema are formed in the region of the macula.

  Guidelines set for the treatment of this affliction recommend that patients should be carefully followed without treatment for 3 - 6 months as in approximately one third of the cases the condition can spontaneously resolve. (Branch Vein Occlusion Study Group, 1984; Krauss and Puliafito, 1995)

  Recommended laser parameters for photocoagulation of the macular edema call for making numerous medium white burns of 100 micrometers diameter surrounding the macular using argon blue-green light. (Krauss and Puliafito, 1995)

- *Senile macular degeneration* - caused by neovascular membranes being formed in the chorioidea. Further damage can be prevented by coagulation with the green line of the argon laser or the red line of the krypton laser. (Neimz, 1996)

- *Retinal tumours* - destruction of the tumour is obtained by converting laser energy into heat.

Vitreous Body

The vitreous body is the transparent gel which has a high water content varying between 98 to 99.7%. It also contains 7g/l NaCl and 0.5 g/l soluble proteins (Neimz, 1996). Many of the inhomogeneities in the fluid do not impair vision as the floating particles can be reabsorbed by biological mechanisms. Major pathologic alterations are due to the formation of new membranes and neovascularizations extending from the retina into the vitreous body. These can be treated with thermally acting lasers due to the vicinity of the retina. Short pulsed lasers evoking photodisruptive effects may only be used for lens surgery and on the front segments of the eye.

Lens

Photodisruption, introduced in the 1980's was first used to treat secondary cataracts non-invasive. (Krauss and Puliafito, 1995) The photodisruptive effects (see section) of Nd:YAG and Nd:YLF lasers can be used to cut within and outside the globe of the eye. (Dankiw et al, 1992) Nd:YAG lasers are used to restored vision when opacification of the posterior capsule occurs in up to 50% of patients after cataract operations.

In phototherapeutic keratectomy the photoablative (see section) effects of the excimer laser are used to remove surface irregularities and superficial scars from the cornea. Photofractive kertectomy uses a similar technique to precisely reshape the corneal surface and correct refractive errors (Dankiw et al, 1992).

Urology

The Nd:YAG laser is the most versatile and preferred laser for use in Urology (Shapshay,1988). The laser equipment is easily adapted to standard cyctoscopes, ureteroscopes, and nephroscopes and can work in a medium of wither water or urine.

The Nd:YAGs deep tissue penetration permits destruction of lesions by thermal coagulation necrosis rather than excision, making it a superior hemostatic device for endoscopic application (Shapshay, 1988).

The most successful uses of the Nd:YAG laser have been in patients with bladder cancer and to a lesser extent, inpatients with transitional tumours of the ureter, and renal pelvis, urethral strictures, bladder neck contractures and carcinomas of the penis (Hofstetter and Frank, 1983).

Laser angioplasty

Laser angioplasty is a process where by the plaque material built up inside artery walls is vaporised. This process was first demonstrated in by McGuff et al (1963) only three years after the first laser was developed and the first intravascular recanalization user laser was reported by Choy who used an argon laser for radiation of thrombolysis in animals. Choy continued this work and in 1983 performed the first clinical coronary laser angioplasty using an argon laser. However there was a high incidence of complications due to the perforation and occlusion of the vessel (Deckelbaum, 1994).

Laser recanalisation of blood vessels is possible with several laser techniques and systems. Argon, Nd:YAG, pulsed dye, Ho:YAG and excimer lasers are used in both preclinical and clinical operations. Delivery systems used include bare fibres, not metal tips, sapphire probes and

"ball tip" fibers however of these the hot metal tip has largely been abandoned. (Absten and Joffe, 1993 p 66)

Neurosurgery

Through various animal experiments beginning as early as 1965 (Earle et al, 1965; Fox et al, 1965) and later clinical trials (Heppner, 1978; Asher, 1978; Takizawa et al, 1980; Krishnamurthy and Powers, 1994) the tissue interaction effects and hazards of laser light on neural tissue have been studied. These initial experiments involved the ruby, argon and CO_2 lasers and today the CO_2 and Nd:YAG lasers have a large place in the tools utilized by the neurosurgeon (Ball, 1990 p166; Absten and Joffee, 1993 p51).

CO_2 laser in neurosurgery

In 1976 in Austria, a CO_2 laser became the first use of a laser to remove a brain tumor. (Tobler and Tew, 1988) Since then the CO_2laser has been widely adopted and due to its efficiency in vaporising it has been successfully used in vaporising meningiomas, acoustic neuromas, and other tumors. Its primarily use is as an ablative tool and adapted to the microscope becomes a precision microsurgical instrument. The area of greatest impact in laser microsurgery is the removal of basal, intraventricular and spinal tumors. (Tobler and Tew,1988)

Nd:YAG laser in neurosurgery

The first clinical neurosurgical application using the Nd:YAG laser was reported by Beck in 1976. (Tobler and Tew, 1988) During the early to mid 1980's several researchers turned toward the laser for shrinking and coagulating arteriovenous malformation, however the reports indicated its usefulness, but there was not total consensus about its applicability. (Fasano et al, 1982; Fasano, 1981 Wharen et al, 1984; Tew and Tobler, 1986; Tobler and Tew, 1988; Samejima et al, 1988). However by the early 1990 dissent and the Nd:YAG laser was reported as "a vey useful adjunct in shrinking vascular tumors and for treating aneurysms and ateriovenous

malformations." (Krishnamurthy and Powers, 1994) Contact probes on handpieces, used while viewing through the microscope, offer a more precise way to use the Nd:YAG for creating hemostasis in small feeder vessels (Absten and Joffe, 1993 p51). The YAG contact probes offer great precision for the removal of tumours on the spinal cord and brain stem (Ball, 1993 p167).

Table D-4 Uses of lasers in neurosurgery

(Krishnamurthy and Powers, 1994)

Laser	**Clinical procedure**
CO_2	Microneurosurgery (tissue ablation for tumor removal, epilepsy, pain surgery)
Nd:YAG (1.06um)	Volume coagulation of vascular lesions (AVM's, vascular meningiomas)
Nd:YAG (1.32um, 1.44um)	Microneurosurgery Tissue ablation for percutaneous discectomy
Argon, KTP	Microneurosurgery (precise microscopic lesions of spinal cord and brainsteam) Endoscopy for coagulation Percutaneous discectomy (KTP only)
Ho:YAG, Er:YAG	Percutaneous discectomy
Gold vapour, argon pumped or KTP pumped dye laser	Photodynamic therapy

Orthopaedics

Orthopaedic surgeons appear to the lay person to use basic equipment, however their sophisticated drills, saws and milling machines have satisfied the needs of the surgeons so well that lasers must offer significant improvements over these tools to be justified. All of these instruments operate in the contact mode and could possible induce severe mechanical vibrations and haemorrhage. Thus it is straightforward to ask whether laser might represent a considerable alternative in orthopaedic surgery.

Bone fulfils three major functions: mechanical support, protection of soft tissues, and a supply of minerals and blood cells. For these reasons the healing time is of great importance however osteotomies (bone excisions) reported in the 1980's suggested that thermal damage to the bone rim caused a delay in the healing process (Gertzbein et al, 1981).

Keller and Hibst (1994) reported a delay of 3 until 4 weeks after CW CO_2 osteotomy and explained this by looking at the charring zones of the bone. However Forrer et al (1993) demonstrated that the wavelength and pulse duration played a significant role in the thermal damage in bone ablation and by altering these factors managed very little thermal damage.

The hardness of bone results from a complex structure of hydroxyapatite, water, soluble agents collagen and proteins. The high water content is responsible for a strong absorption of infrared radiation. Therefore, CO_2, Er:YAG, and Ho:YAG lasers are predestined for an efficient treatment of bone and of these the Er:YAG shows promising results (Keller and Hibst, 1994).

However there are other factors to be considered apart from the effect of the laser light on bone. The ability for fiber optic delivery, to work through fluid (for arthroscopy) and the desired tissue effect (cutting, ablation and vaporisation) must all be assessed as with any other medical area. The uses of lasers in orthopaedics are summarised by Table D-5.

Table D-5 Summary of laser uses in standard orthopaedic procedures

Procedure	Laser	advantages	Disadvantages
Arthroscopy	CO_2	Precise cutting and ablation	No fibre delivery; does not penetrate through fluid thus joint must be gas insufflated
	Nd:YAG (Contact)	Fibre delivery; good cutting	Cannot be defocused for sculpting and vaporisation
	Ho:YAG	Good cutting ablation and hemostasis all through fluid; fiber delivery; it can sculpt, vaporise and shape tissue like no existing tool	
Vaporisation of polymethylmeth-acrylate (PMMA)	CO_2	Atraumatic and less chance of bone fracture	Produces noxious fumes; irrigation necessary
Percutaneous laser disc decompression (PLDD)	Nd:YAG KTP Ho:YAG	Fiber optic delivery under radiological control; treatment with this method may avoid spinal surgery	More research needed

Dentistry and Oral Surgery

Dentistry was the second clinical discipline to which lasers were introduced with Stern and Sognnaes (1964) investigating the possible uses of the ruby laser. However results were not quite as promising as was first thought and research still continues. In conventional dentistry mechanical drills are used to remove the infected and decayed part of the tooth. The use of these drills is painful because of two reasons:- the induced vibrations and the increased temperature due to the friction of the drilling process. The nerves in the tooth are very sensitive to both these phenonomen. It was thought that the non-contact effect of lasers could overcome these points, however the thermal damage is negligible only when using the superpulsed mode of lasers as CW and pulsed lasers still induce extremely high temperatures.

Table D-6 Lasers in dentistry research

Author	Year	Laser	Tissue
Stern & Sognnaes (series of 4 papers)	1964-1969	Ruby and CO_2	Hard dental tissue
Lobine and Fine	1966	Ruby	Hard tissue
Lobene et al	1968	C02	Enamel and dentin
Adrian et al	1971	Ruby	Pulp of teeth
Yamamoto et al	1980	Q-switched YAG	Prevention of caries
Kuroda et al	1984		Tooth enamel

The last 30 years have seen the development of a variety of lasers which allow dentists to cut soft tissue without bleeding, remove caries, cut cavities in teeth, cure composite resins rapidly, weld metals with precision, desensitise teeth, sterilize exposed pulps and tissue surfaces and treat skin lesions (Gillings, 1996). At present this development and research has led to the American Food and Drup Administration clearing the $C0_2$, Nd:YAG, Ho:YAG and argon lasers for soft tissue procedures (Wigdor et al, 1996).

The Nd:YAG, CO_2 and argon lasers are the most commonly used lasers in dental procedures, particularly in soft tissue surgery. This would include gingivectomy, frenectomy, currettage, crown lengthening, lesion removal and gingival hyperplasia. Low power lasers have been available to dentists for reducing pain and promoting wound healing, but without valid supporting research. (http://dentistry.miningco.com/library/weekly/aa051399.htm , 1999)

The erbium:yttrium-aluminum-garnet (Er:YAG) laser can be used to treat cavities in teeth (FDAA approval in 1997). Low power is used for decay removal and higher power to remove enamel and dentin. Air or water spray is applied to the tooth for effective cutting and cooling of the tooth. (http://dentistry.miningco.com/library/weekly/aa051399.htm , 1999) Keller and Hibst (1997)

reported on the use of this laser in dentistry. They stated that 93% of the patients reported little or no pain when used to treat caries. Several studies indicate that the Er:YAG is as efficient as the high-speed drill for cutting tooth structure, however for the most part, it tends to be slower.

Benefits of laser surgery for soft tissue include lessened or no bleeding, precise cutting, bacterial reduction and reduced pain. Compared to scalpel surgery, dental lasers offer improved visibility due to less bleeding and less need for sutures and periodontal packing. Lasers also reduce post-surgical complications, including bleeding, which makes lasers especially appropriate for gingival hyperplasia. In hard tissue the laser appears to be a safe and effective way of removing decay, preparing and etching of tooth enamel (http://dentistry.miningco.com/library/weekly/aa051399.htm, 1999). However, most of these are only *perceived* advantages only reported by users and have not been supported by independent and properly conducted clinical trials (Dankiw et al, 1993 p31).

Photomedicine (including Biostimulation and Photodynamic Therapy)

Whilst laser therapy is a relatively new phenomenon, the use of light as a therapeutic modality predates the earliest records.

The latter part of the 18th and early 19th centuries saw the rediscovery of heliotherapy (after Helios, the Greek god of light, sun and healing) and sunbaths were recommended for scurvy, rickets, oedema, dropsy, rheumatic arthritis and depression. (Baxter, 1999, page 4). It is also interesting to note that in the 1980's ultraviolet therapy have been recommended for seasonal and other effectives disorders (Lewy et al 1982, Hansch et al, 1987, Wehr et al 1988). However only in a few studies could independent research groups verify the results. The studies were done on very few patients and no clinical protocols were established. (Niemz, 1996 and Dankiw, 1993 p32)

In a study by Cambier et al (1996) on low power laser energy applied to burns to stimulate healing reported, "encouraging results have been described in animal experiments…. However it should be put in perspective because of the lack of reproducibility". The conclusion of this study was that no difference could be detected in the healing process of the treated and untreated burn injuries.

However despite these inconclusive results the application of laser for biostimulation involves the greatest numbers of medical lasers users in Australia, with about 2000 in place. These lasers are used by physiotherapists, chiropractors, acupuncturists and general practitioners (Dankiw et al, 1993 p32).

A brief overview of some of the research done in the biostimulation area is provided in Table D-7 and was modified from

Table D-7 Biostimulative effects of Helium-Neon laser

(Niemz, 1996 p 57 ,Table 3.2)

Observation	**Target**	**Reference**
Wound healing	Skin	Brunner et al. (1984) Lyons et al (1987)
No wound healing	Skin	Hunter at al (1984) Strube et al (1988)
Stimulated collagen synthesis	Fibroblasts	Kubasova et al (1984) Boulton et al. (1986)
Suppressed growth	Cells	Quickenden et al (1993)
Pain relief	Teeth	Carrillo et al (1990)
No pain relief	Teeth	Lundeberg et al (1987)

Appendix E Laser Delivery Methods

Direct delivery

Laser pointers, patient positioning lasers and ophthalmic lasers are examples of direct delivery systems. Laser energy is delivered directly form the emitting aperture to tissue, with or without focusing lenses. Output may be controlled by switching the machine on or off. The beam may be steered by hand or by mechanical means.

Articulated Arm

An articulating arm is a series of hollow tubes and mirrors. The laser beam is transmitted through each tube and is then reflected into the next tube by an appropriately angled mirror. This system provides excellent precision, particularly when adapted to an operating microscope. However, the arms are bulky, expensive and require significant maintenance (Reid and Absten, 1995). The limitations of this delivery method are

- Restrictions to 'line of sight' or along straight segments of a delivery path;
- Optical misalignment of the arm; sterilisation procedures (from the manufacturer) must be rigorously observed to avoid damage to lens or mirrors;
- Dust and grease from the hands can adversely affect the optics of mirrors or lenses. (AS/NZS 4171:1994 p 20)

Hollow Flexible Waveguide

These waveguides are long, semi-flexible steel tubes lined by ceramic tile. Laser energy is reflected down the tube by bouncing the beam off the lateral walls. The efficiency of the system is approximately 75% ie about 75% of the input energy is delivered to the target and the system has a maximum of approximately 40 W. The typical waveguides have a 3mm outer sheath diameter, core diameter of 1 mm and length of 40-50 cm. Optically the waveguides function

similarly to the quartz fibre, however they destroy the properties of coherence and collimation thus loosing precision (Reid and Absten, 1995).

Fibre Optic

Laser energy can be focused by a lens into a long thin, flexible, glass fibre coated in opaque nylon or metal. This fibre can transmit visible and near infrared radiation down the length of the fibre and for it to emerge as a *divergent* beam at the fibre tip. (Energy is transmitted along the fibre by means of reflection and hence the property of intense collimation is lost.) The beam diverges at an angle of between 10^{o} and 15^{o} degrees and hence the distance between the fibre end and the target can control different tissue effects. For example when the fibre is held just above the tissue the intensity is sufficient to produce vaporisation whilst withdrawing the fibre one to two centimetres will produce coagulation.

Typically the fibre delivery system is used in conjunction with a rigid or flexible endoscope and according to the type of fibre utilised it is used in either a contact or non-contact mode.

Limitations of this type of delivery method include fibre breakage either due to harsh bending, dropping, cutting or the use of unsuitable endoscopes and damage to endoscopes or metal fittings from inadequate cooling time and firing of the laser whist the fibre in inside the endoscope.

Appendix F Optical Parameters of Laser Tissue Interaction

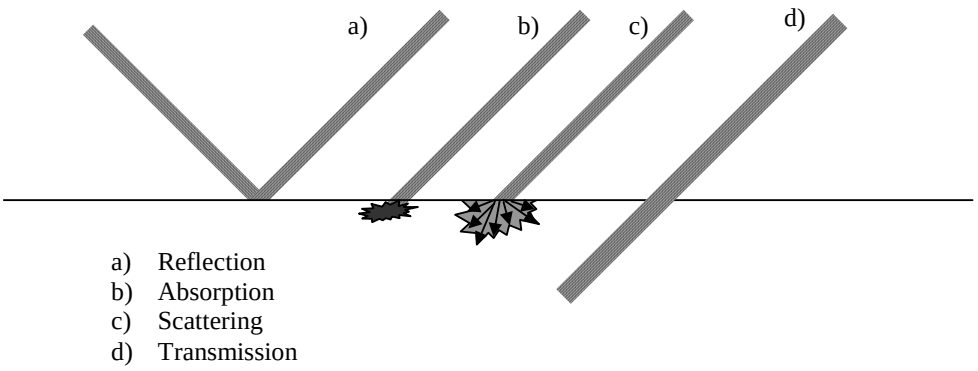


Figure F-1 Optical properties

Reflection

Reflection is defined as the returning of electromagnetic radiation by surfaces upon which it is incident. In general, a reflecting surface is the physical boundary between two materials of different indices of refraction such as air and tissue and the simple law of reflection applies i.e. angle of incidence equals the angle of reflection. When the surface is smooth with surface irregularities being small compared to the wavelength of radiation, the reflected beam is kept intact. This results in *specular reflection* (refer to Figure F-2 (a to (d). However if the surface is rough, as with tissue the incident beam undergoes reflection but the beam is scattered. This is known as *diffuse reflection.* See Figure F-2 (e) on page 205. Specular reflection has the most significance when considering safety issues as unguarded reflections from metal, glass or smooth plastic surfaces can be a major risk to operating staff and patients. In terms of laser-tissue interaction the most important reflection is the diffuse reflection as the surface of the skin and bodily tissues pose a rough surface to the incident laser beam due to the short wavelengths of the laser.

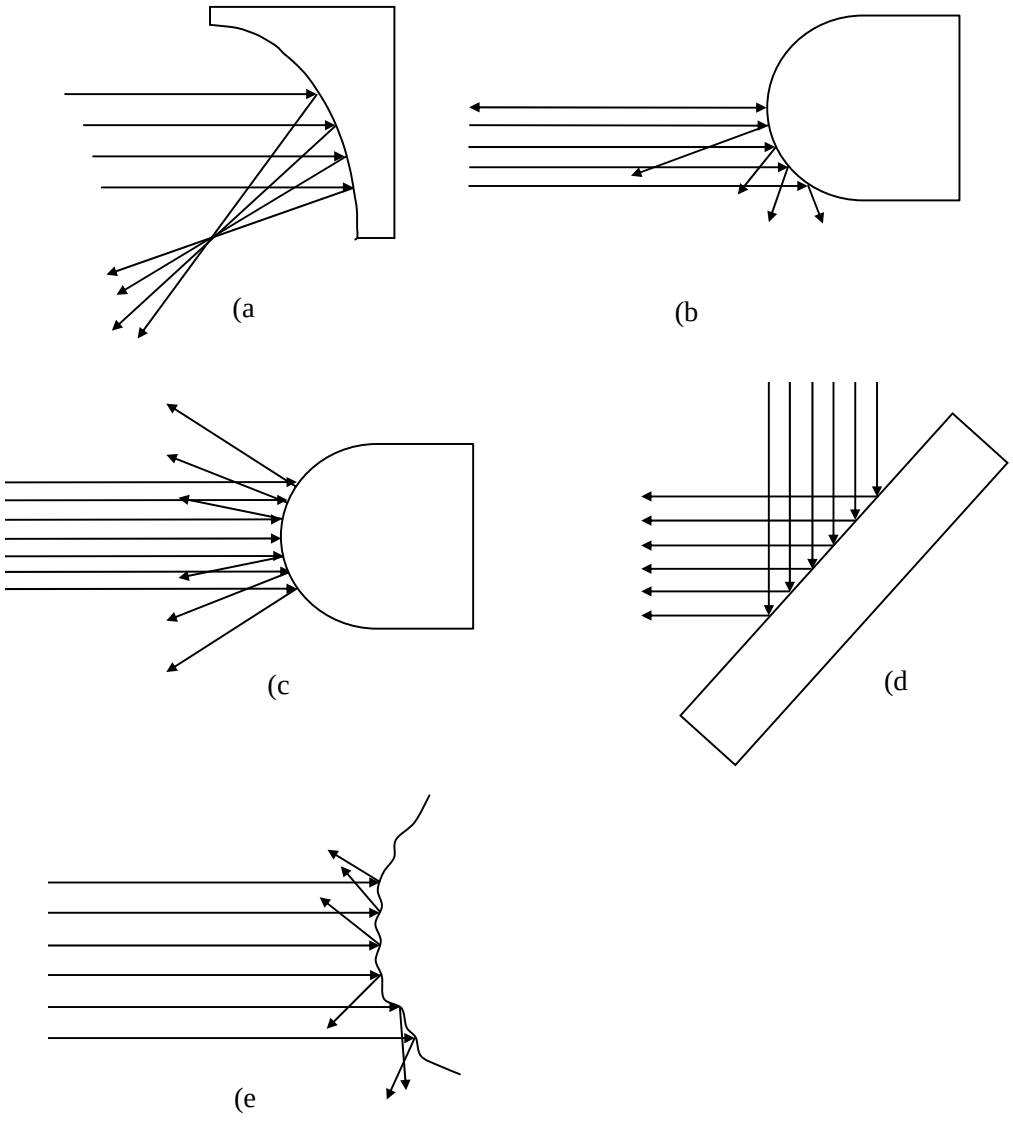


Figure F-2 Reflection

The *reflectivity* of a surface is a measure of the amount of reflected radiation. It is defined as the ration of reflected and incident electric field amplitudes. The *reflectance* is the ratio of the corresponding intensities and is this equal to the square of the reflectivity. Reflectivity and reflectance depend on the angle of incidence, the polarisation of radiation, and the indices of

refraction of the materials forming the boundary surface. Relationships for reflectivity and refraction are commonly known as *Fresnel's Law.*

Scattering

Scattering in tissues may be defined as a change in direction of light propagation and is due to the complex geometry of bio-molecules, as well as to the precise configuration of the interfaces between water and cell or water and organelle membranes. Scattering occurs mainly when absorption is low.

Transmission

Some laser wavelengths can be transmitted through tissue, but have little or no thermal effect. For example the argon laser beam is transmitted through the clear portions of the anterior chamber of the eye to coagulate a blood vessel on the retina.

Absorption

During absorption, the intensity of an incident electromagnetic wave is attenuated in passing through a medium. The *absorbance* of a medium is defined as the ratio of absorbed and incident intensities. Absorption is due to a partial conversion of light energy into heat motion or certain vibrations of molecules of the absorbing material. This thermal damage caused by the laser energy being absorbed by the tissue depends on the wavelength of the beam and the tissue colour, consistency and water content. Heat dissipation depends on the tissue consistency and the blood flow in the surrounding tissue that helps cool the impact site.

The absorption of some lasers depends upon the chromophore (e.g. haemoglobin and melanin) content of the tissue. The wavelength of some beams are greatly absorbed by these chromophores, causing heating of selective tissues. Figure F-3 shows the relationship between laser wavelengths and the absorption coefficients of several tissues and body fluids. These

absorption properties are determined by the amino acids in the mid and far ultra-violet spectrum, chromophores in the visible and near ultra-violet and water with in the infra-red spectrum. See Figure F-2 on page 204.

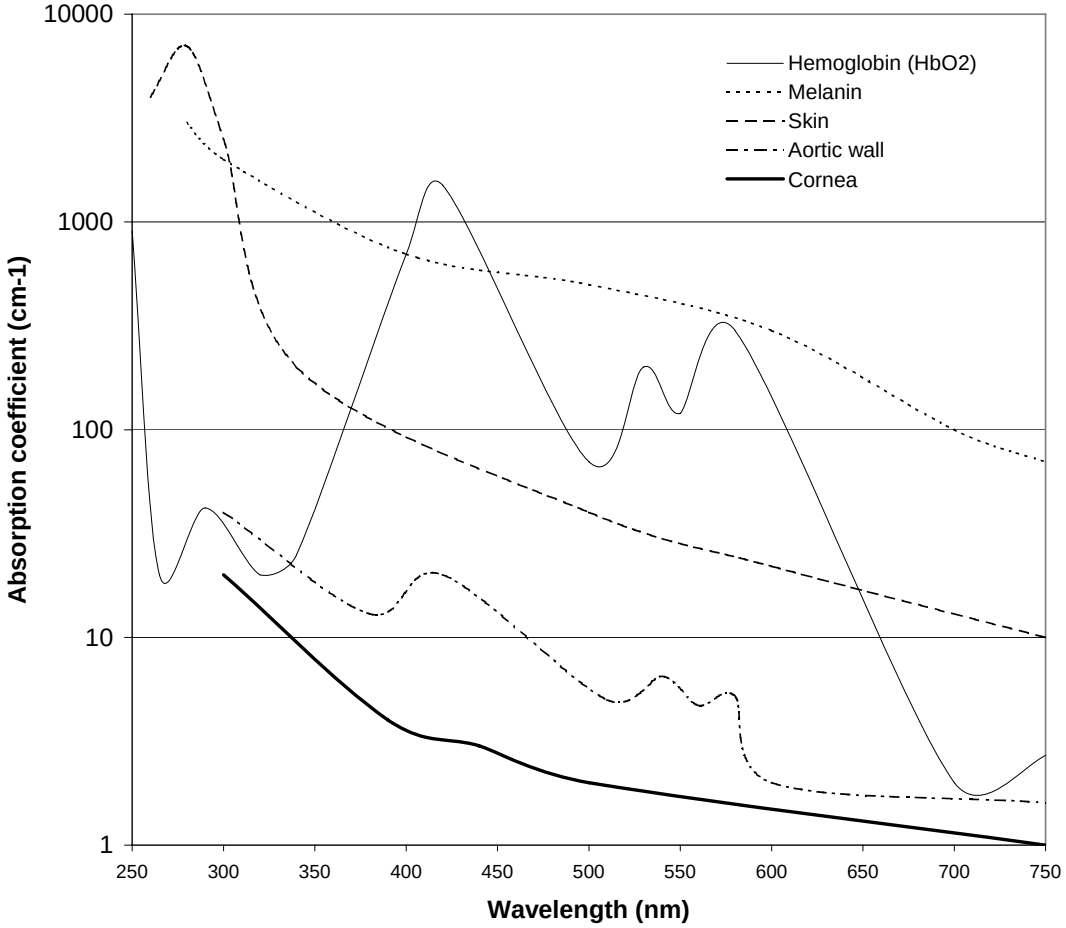


Figure F-3 Absorption coefficients of selected tissues

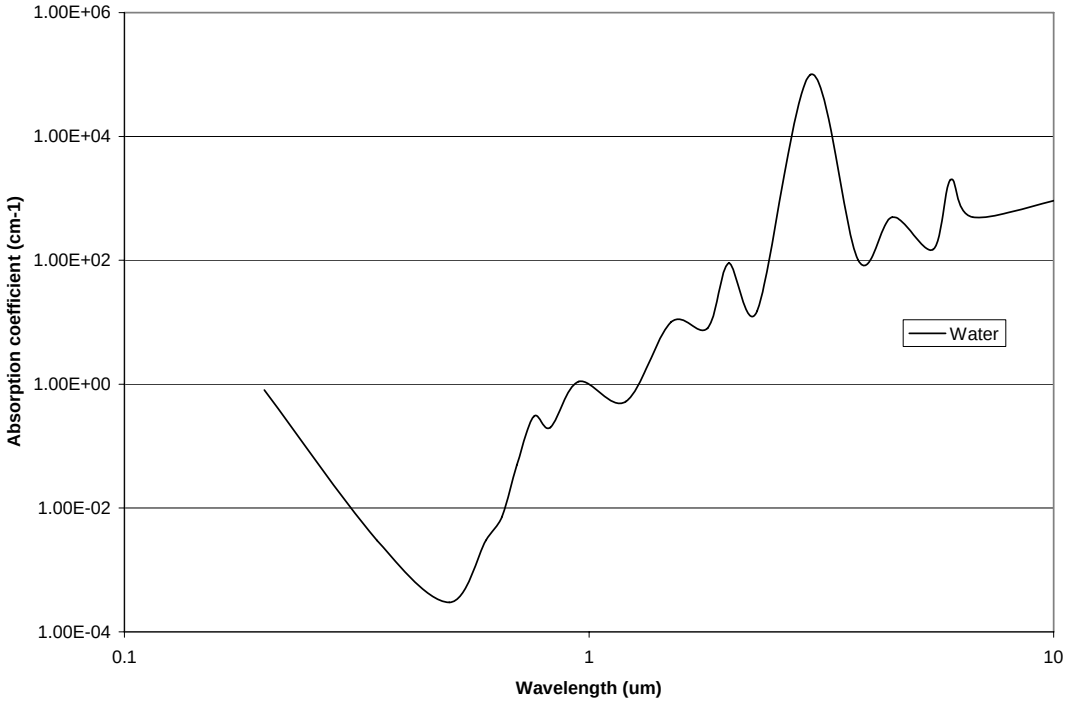


Figure F-4 Absorption coefficient of water

In addition to the above four optical parameters (absorption, reflection, transmission and scattering) the extent of the interaction depends not only on the wavelength but the exposure time, peak power, power density, mode of operation of the device and the nature of the tissue.

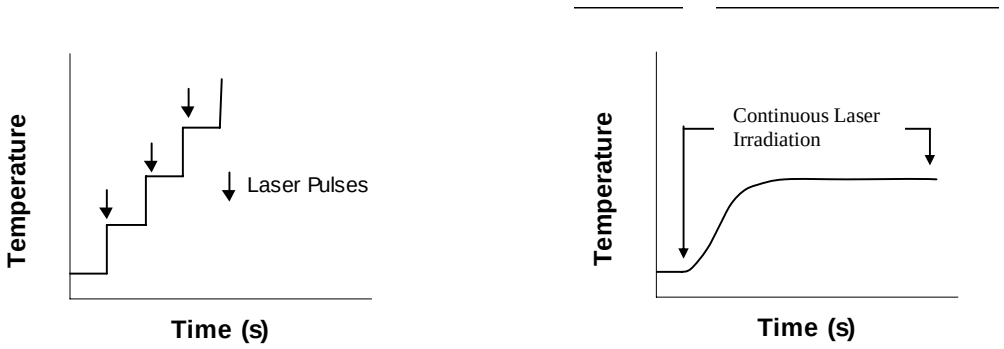


Figure F-5 Temperature variance with mode of laser operation

Appendix G Ethics guidelines and approvals

1. USQ Guidelines for the preparation of applications for ethics clearance

THE UNIVERSITY OF SOUTHERN QUEENSLAND
ANIMAL ETHICS COMMITTEE

GUIDELINES FOR THE PREPARATION OF APPLICATIONS FOR ETHICS CLEARANCE

Researchers seeking permission from the USQ Animal Ethics Committee to carry out experiments involving animals should complete and submit the current ethics approval form, obtainable from the Office of Research and Higher Degrees. In order to minimise delays in the granting of permission to commence work, applicants should read the following guidelines carefully:

1 Ethics clearance is required if the proposed research involves the use of representatives of any vertebrate animal species. No clearance is necessary for research in which invertebrates are the only animals to be used.

2 The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (July 1990), produced jointly by the National Health and Medical Research Council, CSIRO, and the Australian Agricultural Council should be followed in all experiments.

3 Since the University of Southern Queensland does not wish to be associated with illegal or dangerous research projects, final ethics approval will not be granted without receipt of documentation verifying that all necessary licences or other approvals have been obtained. Depending on the nature of the intended research it may therefore be necessary to contact the:

(a) Department of Environment and Heritage (protected fauna)
(b) Lands Department (prohibited species)
(c) Toowoomba City Council (waste disposal or other practices)
(d) USQ Infection Control Committee (if microorganisms are to be used)
(e) Biosafety Committee (genetic engineering) once established in this region
(f) USQ Safety Officer (if chemical or radiation hazards are involved)
(g) any others as specified from time to time.

4 Experiments involving animals will be permitted only if they are essential for the investigation of matters associated with the maintenance and improvement of animal health, welfare, management and production, or with the advancement of medical science. Alternatives to the use of experimental animals are to be employed wherever they will allow the same objectives to be achieved.

5 Investigators are required to exercise responsibility in regard to the welfare of animals they use in their research. They have an obligation to treat the well-being of their animals as an essential factor in the planning and conduct of experiments.

6 Animal experimentation is to be performed only when it can be justified after the scientific and educational benefits of the research have been weighed against the potential harm to the experimental animals.

7 The animals to be used in all experiments must be appropriate for the proposed work in terms of species and strain, age, sex, genetic constitution, and health status.

8 Experimental animals collected from their natural habitats are to be used only if no suitable laboratory-bred individuals are available.

9 Experiments involving animals should be designed to be statistically valid but should employ no more animals than are necessary to achieve the stated objectives. Experiments must not be repeated unnecessarily and the use of death as the experimental end-point is to be avoided as far as possible.

10 The scientific methodology to be used must be the best that is achievable and experimental manipulations performed on the animals are to be carried out only by persons competent to perform them.

11 All experiments must be designed to avoid or minimise pain and distress to the animals. Since there is no certain way to ascertain the degree of pain being experienced by any animal, researchers should perform their experiments on the assumption that the experimental animals they are using suffer pain in a manner similar to humans.

12 In any experiment in which the procedure can reasonably be expected to cause severe pain, the animals should be anaesthetised by a method recognised as appropriate for that species and currently used in veterinary practice. Advice regarding the most appropriate anaesthetic for use on a particular species may be obtained by reference to *Ways of Minimising Pain and Distress in Animals in Research: Practical Information for Research Scientists and Animal Experimentation Ethics Committee,* National Health & Medical Research Council (NHMRC) - Animal Welfare Committee, 1994.

13 Any experimental animal that is clearly exhibiting pain or distress of a kind and degree not predicted in the research proposal must receive immediate relief treatments, even if this means that the experiment must be terminated. If severe discomfort cannot be relieved quickly, the animal must immediately be killed humanely. In those experiments where the inflicting of a certain amount of pain is unavoidable, this must be minimised and the experimental end-point must be as early as possible.

14 No experimental animal should be used for more than one painful or stressful procedure unless there are compelling reasons for carrying out follow-up work.

15 Analgesics, tranquillisers, and neuromuscular blocking agents are to be used in a manner equivalent to what is current in medical practice. However, they should be appropriate for the species involved. Neuromuscular blockers must not be used without simultaneous general anaesthesia unless other humane methods have been used to eliminate sensory awareness. The level of general anaesthesia must be monitored constantly when neuromuscular blockade has been induced.

16 Experimental animals are to be transported, housed, fed, watered, and handled in ways that will minimise distress.

17 Experiments must not commence until written approval has been obtained from the USQ Animal Ethics Committee.

18 Applicants must be prepared to attend an Animal Ethics Committee meeting to discuss their proposals if this proves to be necessary to deal with unresolved issues.

19 Subsequent to ethics approval by the Committee, an applicant must provide written advice to the Committee should there be any variation to the project. In particular, the Committee must be advised immediately of the revision of any techniques.

20 The Committee will request annual progress reports for all applications that receive ethics approval.

21 The Committee reserves the right to undertake spot checks at any time to ensure compliance with ethics approvals. Non-compliance may result in the withdrawal of approval.

2. Ethics application

Please submit this application to the **Manager,** the Office of Research and Higher Degrees

THE UNIVERSITY OF SOUTHERN QUEENSLAND

ETHICS COMMITTEE APPLICATION FOR

ETHICS CLEARANCE FOR

RESEARCH INVOLVING ANIMAL EXPERIMENTATION

1. Having read the document, *Guidelines for Preparation of Application for Ethics Clearance*, applicants should prepare and submit their application to the **Secretary**, Animal Ethics Committee, C/- the Office of Research and Higher Degrees

2. Length of answers and spacing between questions is at your discretion.

2. Typed applications are preferred but if this is not possible, please print legibly

3. If a section is inappropriate, write N/A in the section.

4. Copies of *Ways of Minimising Pain and Distress in Animals in Research: Practical information for research scientists and animal experimentation ethics committee* - NHMRC (Animal Welfare Committee, 1994*)* are available from the Office of Research and Higher Degrees

SECTION A: BASIC INFORMATION ABOUT THE PROJECT

(1) State the title of the project. **Laser Tissue Welding with Bipolar Contact Neodymium: Yttrium Aluminium Garnet Laser (Nd:YAG)**

(2) Give the names, places of work, and contact addresses/telephone numbers of all researchers involved.

Lyn Brodie Faculty of Engineering, USQ 07 46312509

Professor John Grant-Thomson
Faculty of Engineering and Surveying
Ph 46 312519

(3) If the applicant is a higher-degree student, name the proposed supervisor.

Professor John Grant-Thomson

(4) State all departments/facilities, including those not controlled by the University of Southern Queensland, in which the research is to be performed.

Faculty of Engineering and Surveying, USQ
Rooms of Dr G Buckham at St Andrews Hospital, North Street Toowoomba

(5) Give the proposed dates of commencement and completion for the project.

September 1999, March 2000

(6) Indicate the source of financial support the project will receive.
Faculty of Engineering and Surveying

SECTION B: DESCRIPTION OF THE PROJECT *(about one A4 page required)*

Outline the research protocol to be followed, giving sufficient information to allow the Ethics Committee to evaluate what is planned. The following should be included:

(1) the species, numbers and sources of all animals to be used;

Wistar Rats provided by the Faculty of Sciences, USQ
Phase 1 - 5 rats to test base line tensile strength of skin vs orientation and position of test sample
Phase 2 - 2 rats to determine power and time settings of laser for optimum weld strength
Phase 3 - 10 to 15 rats to determine weld strength vs strength of sutured skin

(2) details of what is to be done to the animals, including any proposed surgery or administration of drugs;

In Phase 1 of the experiment the animals will be sacrificed using CO_2 gas as set down by USQ. The skin will be excised and test samples taken and tested for tensile strength. The remains will be disposed of as set down by USQ guidelines.

Phase 2 - Dr Ron Atkinson or Dr Andrew Hoey will provide appropriate anaesthesia for the rats prior to operation. These rats will then have 4 incisions made perpendicular to the spine, approximately 3 cm long. These incisions will be welded using the Nd:YAG laser on varying power and time settings. Stay sutures will

be added to ensure proper healing. After approximately one week these rats will be sacrificed as per phase one and the tensile strength of the wounds tested to determine optimum test parameters.

Phase 3 - Dr Ron Atkinson or Dr Andrew Hoey will provide appropriate anaesthesia for the rats prior to operation.
The rats will then have 2 incisions perpendicular to the spine, approximately 3 cm long. One side will be sutured using standard suture materials and techniques. The other side will be laser welded.
These rats will be sacrificed at set times as per phase 1 and the tensile strength of laser vs sutured welds compared.

(2) what drugs (state dosages and route of administration where possible) or other measures will be used to generate data or to minimise suffering of the animals, both during experimental procedures and while the animals are recovering.

Rats will be anaesthetised with ketamine (Ketamav, 50-100 mg/kg) and xylazine (Rompun, 10-20 mg/kg) as a combined intraperitoneal injection, administered by Dr Ron Atkinson or Dr Andrew Hoey.

SECTION C: JUSTIFICATION OF THE PROJECT

Answer the following questions:

(1) What is the ethical and scientific justification for carrying out the proposed research?

Tissue welding is a generic term that is also referred to as tissue fusion and vessel sealing. The process uses laser energy to activate photothermal of photochemical bonds, however the exact mechanism is poorly understood. Lasers have the advantage over other energy sources e.g. radio-frequency and microwave as they provide the ability to accurately control the volume of tissue that is exposed to the activating energy.

The tissue welding process is a potentially important biomedical application of laser technology and the use of laser energy to effect a tissue weld of clinically acceptable tensile strength is now well documented. However the majority of this work has been done for anastomosis.

Lasers used for vascular anastomosis include CO_2, argon, diode, and Nd:YAG and a variety of power and temperature settings. However there has been little work done on the welding of cutaneous tissue. In addition to this there is no current literature available on the use of the bipolar contact Nd:YAG laser. Thus research will provide valuable data, not only on the base line tensile strength of rat skin (which are often used in tissue welding studies) but on the results of the suitability of this particular laser for welding skin.

(2) Why is it not possible to achieve the desired objectives without the use of experimental animals?

The exact mechanisms of tissue welding is not fully understood and the current algorithms for the predicting of temperature in skin/tissue due to laser radiation are not valid for a contact laser. Thus the temperature of the skin/tissue cannot be accurately predicted using any computer models and the effect that this temperature will have on the tissue or the subsequent healing process.

(3) What is the justification for using the animal species chosen?

The rats are well known for their rapid healing and can be currently cared for in the university.

(4) What has been done to ensure that the proposed experiments are statistically valid but will use no more animals than necessary? State the source of your statistical advice.

It is proposed to minimise the number of variables and thus minimise the number of animals used. The rats will be matched for age, sex and general health. Strength of both skin and welded skin will be carried out in one orientation and at one elongation speed.

Phase 1 - Haut RC in a 1989 study on the orientation and location on the strength of rat skin tested 36 rats. However variables included location, orientation (parallel and perpendicular to the spine) and elongation speed in the tensile tests. Whilst in this phase of the experiment I will analyse the tensile strength of the skin from different locations however the orientation (parallel to the spine only) and elongation speed of the test will be constant. In addition to this a small test specimen (micro-tensile test die ASTM D-1708) will enable a larger number of test specimens to be taken from the one animal.

Phase 2 - The optimum temperature for welding is non specific and the exact mechanisms are not well understood but it is believed to rely on heat-induced alterations in collagen of the tissue. (Neimz, 1993; Welch and Van Gemert, 1993). Collagen denatures at approximately 80 0C and at this point the tissue matrix is eliminated and the process of scarring becomes apparent. The principle of coagulation which is used in hemostasis, tissue welding and induction necrosis in small tumors takes place at a temperature of between 50 and 80 ^{0}C. The tissue temperature achieved by laser radiation depends on the absorption coefficients of tissue photoacceptors (chromophors, water and protein) and its interaction with the specific wavelength of the laser and the mode of operation of the laser (continuous or pulsed). There is currently no data for the mode and power settings of a **contact** laser and the corresponding tissue temperature, thus this phase will allow different settings to be evaluated and the corresponding temperature and effectiveness of the welding process to be evaluated.

The temperature will be monitored both by infrared thermometer and a small bead thermistor inserted in the tissue.

Phase 3 - The number of rats needed in this phase may vary depending on the results of phase 1. This will determine the number of test samples that can be taken from one welded incision.

The current medical literature favours small group sizes indicating small variances. Francis MAJ et al (1996) investigated the use of CO_2 and argon lasers for tendon repair. The study design used 40 Spraque Dawley rats divided in to 4 groups, giving n= 10; Tayor DL et al (1997) used a group of 36 rats divided in 3 groups, giving n= 12.

I propose to use slightly larger sample sizes by taking a number of samples from the one animal. If the results of phase 1 indicate little or no variation in the tensile strength of skin parallel to the spine (and perpendicular to Langer's Lines) then approximately 3 test samples can be taken from each welded incision.

- Matthew DE ,Farewell VT;1996: Using and understanding medical statisitics. Karger New York, 3rd, revised edition
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- Francis MAJ, Kilkelly X, Theodore CPT et al (1996) Tendon repair by laser welding: A histologic and biomechanical comparisons and suture repair with c02 and argon lasers. Lasers in Surgery and medicine. 19:487-491
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- Haut RC. (1989) The effects of orientation and location on the strength of dorsal rat skin in high and low speed tensile failure experiments. Journal of Biomechanical Engineering 111:136-140, 1989

(5) What evidence is there that the project has a reasonable chance of achieving its objectives?

In an earlier pilot study approximately 18 months ago, rat skin was successfully welded. It is now proposed to scientifically test the strength of these welds.

SECTION D: PAIN AND DISTRESS

Describe and justify as appropriate

(1) the measures that will be taken to monitor the experimental or trapped animals for evidence of accidental injury, pain or distress;

Animal will be cared for in the USQ animal house and monitored.

(2) any instances in which a particular experimental animal will be subjected to more than one painful or stressful procedure;

N/A

(3) the post-operative care that will be used after any surgical procedure has been performed on the experimental animals;

Animal will be cared for in the USQ animal house and monitored. If the welding process is not successful the animals will be anaesthetised and re sutured using conventional techniques.

(4) the methods to be used to identify individual animals, especially if potentially stressful procedures such as tagging, ear-punching, tattooing, or banding are proposed;

caged numbered and skin marked with permanent pen.

(5) what autopsy or other procedures will be performed when animals die unexpectedly.

As the procedure only inflicts skin wound on the animal it is not expected that they should die from the procedure.

SECTION E: ADEQUACY OF STAFF AND FACILITIES

Provide evidence that

(1) the facilities to be used are adequate to allow the project to proceed to its expected conclusion;

- Separate lab space within the Faculty of Engineering has been set aside.
- New Tensile testing equipment, Hounsfield H50K-S Materials Testing Machine, ultralight grips, and 250N load cell have been purchased by the Faculty of Engineering.
- Supply and monitoring of rats, along with appropriate medical/veterinary advice have been sought and granted from the Faculty of Science
- Use of the laser system has been granted by Dr G Buckham and Dr Ross Harrington, who will also give medical advice and assistance i.e. suturing of the animals

(2) the staff involved are competent to do the proposed work;

2 specialist medical practitioners, who will assist with medical techniques and advice, will supervise me. I currently hold a Bachelor of Engineering (Electrical and Electronic)

(3) adequate space, technical support and feeding/watering materials are available in the USQ Animal House if the experimental animals are to be housed there.

Advice has been sought from staff of the animal house. At any one time there will not be a large number of rats needing care, it will pose no problem.

SECTION F: KILLING AND DISPOSAL OF EXPERIMENTAL ANIMALS

State:

(1) the procedures that will be used to kill the animals humanely once they cease to be useful and name the person who will do the killing;

The rats will be gassed in CO_2, in accordance with USQ guidelines and this will be done by myself under instruction by Dr Ron Atkinson.

(2) the method of disposal of dead animals or animal tissues at the conclusion of the project;

In accordance with USQ guidelines.

(3) whether or not it is not feasible to dispose of the animals in a healthy state.

N/A

SECTION G: OTHER APPROVALS

Provide a copy of all licences or other authorisations to collect, use and dispose of the experimental animals that will be used in this project.

See attached letters

I have read the *Guidelines for Preparation of Applications for Ethics Clearance* and agree to conduct my work in accordance with the principles detailed in that document.

Signed: _____ Date: _____

Noted by Head of Department: _____

3. *Ethics committee approval*

USQ
TOOWOOMBA

THE UNIVERSITY OF
SOUTHERN
QUEENSLAND

TOOWOOMBA QUEENSLAND 4350
AUSTRALIA
TELEPHONE (076) 31 2100

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rh:lcletters

8 July 1997

Mrs Lyn Brodie
C/- Faculty of Engineering and Surveying
USQ
TOOWOOMBA QLD 4350

Dear Mrs Brodie

Re: Ethics Clearance for Research Project, *Tensile Testing of Welded Skin*

Further to my correspondence dated 26 April 1997, I wish to inform you that advice has recently been recently from St Andrew's Hospital regarding the conduct of your experiments on the Hospital grounds. The Hospital's institutional Ethics Committee has considered and approved the project. In addition, Queensland Health has approved the conduct of the project. A copy of their advice forwarded to St Andrew's Hospital Toowoomba is attached for your information.

I am now able to confirm the University's ethics approval for your project and advise that reference number **97REA020** has been assigned to this approval.

May I remind you of details included in the, *Guidelines for the Preparation of Applications for Ethics Clearance.*

- Subsequent to ethics approval by the Committee, an applicant must provide written advice to the Committee should there be any variation to the procedures. In particular, the Committee must be advised immediately of the revision of any techniques.

- The Committee will request annual progress reports for all applications that receive ethics approval.

- The Committee reserves the right to undertake spot checks at any time to ensure compliance with ethics approvals. Non-compliance may result in the withdrawal of approval.

Yours sincerely

RUTH HILTON
Manager
(Research and Higher Degrees)

enc

4. Queensland Government approval

QUEENSLAND HEALTH

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147-163 Charlotte Street
Brisbane Q 4000

Postal GPO Box 48
Brisbane Q 4001

Phone (07) 3234 0111
Fax (07) 3221 0951

Enquiries to Yvonne Orley
Telephone (07) 3234 1072
Facsimile (07) 3221 7535
File Number PHM4-S3
Our Reference
Your Reference

3 June 1997

Luke G Hughes
Chief Executive Officer
St Andrew's Toowoomba Hospital
PO Box 263
TOOWOOMBA Q 4350

Dear Mr Hughes

SUBJECT: LASER RESEARCH PROJECT PROPOSAL

Thank you for your correspondence of the 29 May 1997, which states that the Board of Governors of St Andrew's Hospital approves the conduct of the above project on the hospital grounds and the Hospital's institutional Ethics Committee has considered and approved the performance of the project.

Whilst the Chief Health Officer is supportive of the need for support for research within both the public and private sector, and acknowledges that usually this nature of research is conducted within the parameters of a university, the local difficulty is recognised (including the understanding that the equipment which is integral to the project is unable to be moved to the university).

The Chief Health Officer wishes to advise that an approval is given for the use of the specified rooms within the premises licensed under, Licensed to Use No. 175, for the purpose of a research project, "Tensile Testing of Welded Skin"' to be conducted under the supervision of Professor Grant - Thomson and Dr Ross Harrington.

Approval to use the designated area for the animal research project would be contingent upon:

- maintaining patient safety (and the integrity of the service provision);
- addressing human and animal ethics in relation to the proposal; and
- meeting relevant research standards; and that
- approval also does not set a precedent for further research of this nature.

It is noted that relevant documentation within and subsequent to the proposal, has been received which addresses the above. If you have any further queries relating to this issue, please contact Ms Yvonne Orley, (Project Officer) on (07) 3234 0172.

QUEENSLAND GOVERNMENT

Dr Diana Lange
CHIEF HEALTH OFFICER

5. St Andrew's Hospital approval

ST. ANDREW'S
TOOWOOMBA
HOSPITAL
280 North Street Toowoomba Queensland
PO Box 263 Toowoomba Qld 4350
Telephone (076) 31 4666
Facsimile (076) 33 4059

29 May 1997

Dr R Atkinson
Chairman
USQ Animal Ethics Committee
The Office of Research and Higher Development
University of Southern Queensland
TOOWOOMBA QLD 4350

Dear Dr Atkinson

PROPOSED LASER RESEARCH PROJECT

The Board of Governors of St Andrew's Hospital approves the conduct of the above project on the Hospital grounds, more specifically including use of Dr Geoffrey Buckham's suite. The Hospital's institutional Ethics Committee has considered and approved the performance of the project.

Yours sincerely

LUKE G HUGHES
Chief Executive Officer

Appendix H Certificate of equipment Calibration

HOUNSFIELD TEST EQUIPMENT
CERTIFICATE OF CALIBRATION

6 Perrywood Business Park
Honeycrock Lane, Salfords
Redhill RH1 5DZ. ENGLAND

Telephone: (01737) 765001
Telex: 262982 HTE G
Fax: (01737) 764768

Date of issue 27/08/99
Certificate No 5420P99
Page 1 of 1
Approved Signatory
O Davies

LOCATION SOUTH QUEENSLAND UNIVERSITY

LOAD CELL ONLY S-SERIES

LOAD CELLS 250N Serial No. 0144410

DATE OF VERIFICATION 27th August 1999

This certificate complies with HMC 4.1, HMC 4.2a, and HMC 5.2

We Certify that the above noted Load Cell(s) have been calibrated and verified. The Load Cell output was found to indicate better than ±0.5% deviation from the true applied force within the range of 2% to 100% of full scale.

The True Force and the Indicated Force methods were used

Instrument temperature 24°C

The following verification devices were used, these are traceable to the National Physical Laboratory.

Load Display Unit LDU-02

Load Cell Ser No D14845 Ref Sheffield 983325
Certified Masses Ref ATG/MBM/94

This certificate is valid for twelve months from the date of verification

Owen Davies

Glossary of terms

Ablation	Volume removal of tissue by vaporization
Absorption	Uptake of light energy by tissue, converting it into heat
Accessible Emission Limit (AEL)	refers to the amount of laser radiation emitted by the laser and which is collected by a detector with a specified aperture.
Active Medium (or Lasing medium)	The lasing medium of the laser determines the wavelength of the emitted light and provides the optical gain in the laser. This active medium can be a solid, liquid, gas or semiconductor (see section)
Apoptosis	Programmed cell death
Biostimulation	
Blunt Dissection	The process of using forcepts, fingers or scissors (in the oppositer way to normal) to tear connective trissue strands to free organs without damaging blood vessels or nerves. A scalpel is then reserved for cutting the skin and dense layers of fascia. See disection and disection equipment
Cautery	Achieving hemostasis of bleeding vessels usually by heat from laser or electrosurgical units
Central vein occlusion	Blockage of the vein as a consequence retinal veins become dilated and severe edema are formed in the region of the macula.
Chromophore	Optically active material in tissue which acts as the target for laser light
Coagulation	Destruction of tissue by heat without physically removing it
Coherence	Wave patters in phase in time and space

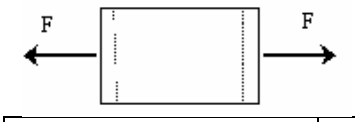
Coherent	the atoms are stimulated to emit light in phase with the incident light wave and the phase is maintained over many cycles. This coherence has an additive effect on the amplitude or power.
Continuous wave (CW)	Constant, steady state delivery of laser power
Diabetic retinopathy	Retinopathy is a non-inflammatory eye disorder caused by changes in the retinal blood vessels. Due to lack of oxygen new blood vessels are formed and if these vessels haemorrhage complete blindness can follow.
Diffuse reflections	Reflections coming from a rough reflecting surface which reflects light randomly in all directions and does not maintain internal order in a light beam.
Dimer	Two of the same thing
Directional (or collimated)	the light emitted is a relatively narrow beam in a specific direction. This property can be contrasted with the pattern of ordinary light, whose beam diverges and spreads out as it travels away from the source. This collimated property of a laser beam is extremely important as it minimises any loss of power. When this collimated laser energy passes through a lens, the beam can be focused into a pinpoint spot to allow a concentration of power and precision.
Dissection Equipment:	**scalpels** (small and large)**, blunt-ended curved scissors, and forceps** (preferably with rounded points). See blunt disection and disection
Dissection	The process of freeing the organs from fascia (fibrous connective tissue) and demonstrating the variations in its density. See also Blunt dissection and disection equipment.

Electromagnetic spectrum	The "electromagnetic spectrum" is a term used to describe the entire range of light radiation, from gamma rays to radio waves. We tend to think of optical radiation as "light," but the rainbow of colours that make up optical or "visible" light is just a tiny part of a much broader range of light energy. Many of these other portions of the spectrum get totally or partially blocked by earth's atmosphere, requiring telescopes to be flown in space if we are to observe objects at these wavelengths. There are no hard boundaries or breaks in this distribution of light energy, although for convenience we assign names to various sections, as shown in the diagram above.
Excimer	Word from organic molecular physics which means excited dimer
Excitation Mechanism	The *excitation mechanism* provides a source of energy that excites or "pumps" the molecules, atoms or ions of the lasing medium to a higher energy state. This mechanism can be optical as in the case of solid-state lasers; electrical as with gas lasers, mechanical or chemical.
Fabry-Perot cavity	Gain in a laser is increased by placing the active laser medium in between two mirrors which face each other. This arrangement is known as a Fabry-Perot interferometer or a Fabry-Perot etalon.
Feedback mechanism	See Fabry-Perot cavity and resonator
Fresnel reflections	These are partial reflections of light from smooth surfaces of transparent materials such as glass. Fresnel reflections have the same characteristics as specular reflections, but the power possessed by the reflected light is considerable reduced in magnitude.
Hemostasis	The ability to stop bleeding

Human Aversion Response	A natural protective instinct which protects the eye from visible light and infra-red wavelengths. This causes the lid reflex (blinking) to occur between 0.2 and 0.25 seconds, but this may not be fast enough to prevent injury from the intense light generated by a laser, and will not it protect from UV wavelengths.
Hysteroscopy	Direct visual inspection of the cervical canal and uterine cavity through a hysteroscope.
Langer's Lines	Langer's lines are lines of tension or cleavage within the skin that are characteristic for each part of the body. In microscopic sections cut parallel with these lines, most of the collagenous bundles of the reticular layer are cut longitudinally, while in sections cut across the lines, the bundles are in cross section. The cleavage lines correspond closely with the crease lines on the surface of the skin in most parts of the body. These cleavage lines are of particular interest to the surgeon because an incision made parallel to the lines heals with a fine linear scar, while an incision across the lines may set up irregular tensions that result in an unsightly scar. In other areas of the body Langer's lines are visible or can easily be seen by compressing the skin.
Laparoscope	A type of endoscope, consisting of an illuminated tube with a optical system that is inserted through the abdominal wall for examining the peritoneal cavity.
Lasing Medium (or active medium)	The lasing medium of the laser determines the wavelength of the emitted light and provides the optical gain in the laser. This active medium can be a solid, liquid, gas or semiconductor.
Maximum permissible exposure (MPE)	The level of laser radiation to which a person may be exposed without hazardous effects of adverse biological changes in the eye or skin. MPE levels are determined as a function of laser wavelength exposure time and pulse repetition.

Mode	A term used to describe how the power of a laser beam is distributed within the geometry of the beam. Also used to describe the operating mode of a laser such as continuous wave (CW) or pulsed.
Monochromatic	Light that has only one wavelength as apposed to white light which is a combination of many wavelengths (or colours). The wavelength of the laser and hence its position in the electromagnetic spectrum is specific to and determined by the lasing medium.
Necrosis	Cell death
Nominal hazard zone (NHZ)	The space where any laser radiation, direct, reflected or scattered is outside the MPE levels. Safety measure are required by everyone inside this area.
Otorhinolaryngology	The area of medicine concerned with the diseases of the ear, nose and throat.
Output Coupler	Partially transparent mirror at one end of the Fabry-Perot cavity to allow the laser beam to escape the feedback mechanism. See also resonator.
Papillomatosis	Benign tumors, nipple like in development
Photocoagulation	Tissue coagulation caused by laser light
Photodisruption	Creating an acoustical shock wave through Q-switching or mode-locking to "snap" cell membranes
Polymethylmetha-crylate (PMMA)	Bone cement used to stabilize a prosthetic in arthritic joint replacement. Prosthetic may be metal or plastic.
Population inversion	Occurs when a substance has been energised or excited so that more atoms or molecules are in a higher excited state than in a resting state.

Q-switching	Switching of the resonator producing very high peak powers for very short bursts
Resonator	A resonator can be broken down into a feedback mechanism and an output coupler. In most cases this is some form of a Fabry-Perot cavity which allow stimulated light to bounce back and forth through the lasing medium (the feedback mechanism). Usually one of the mirrors is totally reflective and the other is partially transparent to allow the laser beam to escape (the output coupler).
Retinal detachment	The separation of the retina from the chorioidea in the back of the eye and can often occur when retinal holes are undetected.
Retinal hazard region	The wavelengths in the visible and near infrared spectrum which are transmitted through the eye fluid to the retina. (400-1400 nm)
Specular reflection	Reflection of light occurring at a smooth, mirror-like surface. This type of reflection changes the direction of the light, but does not change characteristics of the beam itself.
Spot size	The mathematical measurement of a focused laser spot. This indicates the "optical spot size and not the "impact size" i.e. the size of the impact on the tissue
Strain	Is a measure of the distortion resulting from a tensile force or stress



Stress (tensile)	Tensile stress is applied to a body when equal and opposite forces are exerted on the ends of the test specimen along the same line of action. As seen in the Figure 1 Stress is given by $\frac{F}{A}$ where F is the Force in Newtons and A is the cross sectional area in square meters (m^2)
Superpulse	An operating mode of the laser. The output pulsing 250-1000 times per second, with peak posers per pulse higher that the maximum attainable in the continuous mode
Tensile Force	Forces which produce tensile stress (See Stress (tensile))
Thermal recovery time	The time required for heat to dissipate after a laser pulse is delivered. It represents a limit to how frequently the laser pulses can be repeated without heat build up in the tissue and increase in thermal damage.
Thermal relaxation time	A time constant that is the amour of time required for thermal energy to diffuse to surrounding tissues, thus heating and injuring them.
Tissue welding	generic term that is also referred to as tissue fusion or vessel sealing. The process uses laser energy to activate photothermal or photochemical bonds.

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