Investigation of Infrared Neural Stimulation in the Cochlea

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by

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Abstract

The cochlear implant is a highly successful neuroprosthetic device, used by hundreds of thousands of patients world wide. One limitation of the device is the spread of electrical current in the cochlea, preventing strong localisation of neural stimulation. This spread of electrical current limits most patients’ ability to understand voice in a noisy environment. Recently, a number of optically driven neuronal stimulation techniques have been developed. These include infrared neural stimulation (INS) which uses short pulses of infrared light to trigger electrical responses in neurons. Studies on INS in the cochlea and other neural targets show improved spatial localisation of stimulation compared to electrical stimulation, suggesting that the technique may be beneficial for use in implants. This thesis investigates the feasibility of INS for use in cochlear implants through development of a combined Monte Carlo and finite element model, together with acute experimental studies in the guinea pig cochlea.

To achieve stimulation with typically reported pulse characteristics (i.e. a single 25 µJ pulse) the combined Monte Carlo and finite element model predicts temperature rises in the cochlea to be on the order of 0.1 °C. When INS is used to stimulate peripheral nerves, the required temperature increase is greater, on the order of 1 °C to 10 °C, depending on the pulse energy, wavelength and neural target. Analysis of the spatial behaviour of heating suggests that the heat remains localised to the area directly exposed to the laser irradiation. This prediction lends support to the experimental results showing strong spatial localisation of stimulation. Applying the model to multiple 25 µJ pulses, at a stimulation rate of 250 Hz, suggests that the temperature increase is only 2.3 °C. When using multiple independent emitters, this increases further, to a temperature increase of 2.8 °C with an emitter separation of 750 µm and 4.4 °C at 250 µm. These predictions suggest that INS is feasible for use in cochlear implants without greatly increased thermal load compared to a single emitter. When applied to in vitro studies, the modelled behaviour of heat distribution and flow during INS may assist in understanding the biophysical mechanisms behind
Experimental results for infrared stimulation the cochlea, suggest that the target of stimulation may be hair cells, rather than spiral ganglion neurons as generally suggested in the literature. Infrared stimulation results presented in this thesis were unable to elicit a clear response after chemically deafening the cochlea with aminoglycosides, whereas a response could be observed in normal hearing animals. Although the lasers used for INS generate an audible click, a purely optoacoustic mechanism is not consistent with all of the previously reported results. Instead, a direct interaction between the infrared light and residual functions of the hair cells may also play a role in the response observed. Further studies would help to demonstrate whether these two mechanisms can combine to give the response seen with infrared stimulation of the cochlea. Combining electrical and optical stimulation gave a 8 – 10% reduction in electrical thresholds, even in profoundly deaf animals. Given the power requirements of currently available laser sources, it will require significant optimisation of the process to provide any clinical benefit.

Overall, it appears unlikely that INS will provide a revolutionary improvement in cochlear implant performance as current results may rely on residual hair cell functionality. However, the interaction between infrared light and hair cells may allow a number of new scientific techniques to be developed. For example, there may be some potential to use of infrared light to directly stimulate hair cells in patients with residual hearing.
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Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma and to the best of my knowledge contains no work previously published or written by another author except where due reference is made in the text of this thesis.

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Artificial stimulation of neurons to replace damaged neurons or sensory systems, or the development of neuroprosthetics, has arguably been one of the key goals of scientific research for over a century (Navarro et al., 2005; Scott, 2006). Replacement of lost hearing has arguably seen the most success in this field with use of artificial stimulation, leading to a multimillion dollar industry and a number of implant manufacturers (Clark, 2003). Ongoing research into improved neural stimulation techniques has uncovered a number of optically based techniques, which offer improved specificity of stimulation. This thesis investigates the application of Infrared Neural Stimulation (INS) in relation to the the cochlea, and potential applications to the highly successful cochlear implant.

This chapter provides a brief introduction to to the neural system in Section 1.1, for readers without a background in neurophysiology; the cochlea and current status of the cochlear implant in Section 1.2; optical techniques for activation of neurons Section 1.3; current applications of Infrared Neural Stimulation in 1.4; and an outline of the thesis structure in Section 1.5.
Chapter 1. Introduction

1.1 Nervous System

The nervous system is a highly specialised communication network which acts as a control system for the body. A full description of this system is out of the scope of this thesis, but a brief primer on it is presented here for readers unfamiliar with it. For a thorough treatment, readers are referred to Kandel et al. (2000), which this primer is based on.

Although the nervous system comprises only 3% of the body’s weight it is the most complex system in the body. The nervous system allows for fast processing of information and short reaction times. Unlike more regulatory systems, such as the endocrine system, the nervous system reacts in the order of milliseconds. The nervous system includes the neurons or neural tissue along with neuroglia, support cells, which support the neurons by insulating and protecting them and promotes efficient signalling (Kandel et al., 2000).

The nervous system is anatomically divided into the central nervous system (CNS) and peripheral nervous system (PNS). The CNS consists of the brain and spinal cord, which are encased in the skull and vertebral column respectively. It processes information and acts as a control system for functions in other parts of the body. The PNS comprises all of the neural tissue that is not part of the CNS. The PNS is broadly divided into afferent and efferent divisions. The afferent division receives sensory information from peripheral tissue and organs and delivers it to the CNS, while the efferent division carries motor commands to muscles from the CNS. Peripheral nerves which make up the PNS are comprised of bundles of axons with connective tissues and blood vessels. (Kandel et al., 2000)

1.1.1 Neurons

Neurons are the main signalling unit of the nervous system. They carry electrical signals, called action potentials, down long branches of neurons, or axons, before connecting to another neuron to transmit the signal. By having multiple inputs, neurons are able to process information in addition to simply transmitting it. Although there are a large range of neuron types in the body, all share four distinct morphologically defined regions, as shown in Figure 1.1: the cell body, dendrites, the axon and pre-synaptic terminals (Kandel et al., 2000). The cell body (soma) is the main part of the cell and contains the nucleus along with performing much of the metabolic activity of the cell. Dendrites and axons are branches from the main cell body. Dendrites spread out from the cell body and are the primary part of the cell that receives signals from other neurons. Axons, unlike dendrites, extend away
from the cell body and carry action potentials to other neurons. Axons can be from 0.1 mm to 3 m in length and carry information at rates of up to 100 m.s$^{-1}$. Action potentials are binary, transient, electrical impulses, with an electrical amplitude of approximately 100 mV and duration of roughly 1 ms. The rate of conduction for action potentials can be increased when axons are wrapped in myelin by neuroglia. This sheath of myelin is broken at regular intervals by the nodes of Ranvier. Towards the end of the axon, it branches to allow for communication with multiple neurons. The point where the axon ends and meets another neurons is the synapse. The synapse connects the axons from the presynaptic cell to dendrites of the postsynaptic cell (Kandel et al., 2000).

Action potentials are sometimes described as electrical impulses travelling along an axon, while this coarsely describes the behaviour and process, the actual biophysical mechanism of an action potential is more complex. Rather than being charge running down an axon, an action potential is a change in cell membrane potential which runs down the axon. When resting, neurons maintain a transmembrane potential of approximately $-70$ mV, although this value depends upon the cell type. This potential is due to a difference in ion concentration between the intracellular fluid and extracellular fluid and is maintained by ion pumps on the membrane, as shown in Figure 1.2. The ion pumps keep a higher concentration of potassium ions ($K^+$) in the intracellular fluid and higher concentration of sodium ($Na^+$) and chlorine ions ($Cl^-$) in the extracellular fluid. This active movement of ions creates both an electrical gradient, where the inside of the cell is at a lower potential than outside, and a chemical gradient, with different concentrations of ions in the intracellular fluid and extracellular fluid. These gradients give rise to a chemical diving force and an electrical driving force. The chemical driving force depends upon the relative concentrations of ions on either side of the membrane and ions tend to move from the more concentrated side to the less concentrated side. This results in potassium ions being driven outside the cell, while sodium and chlorine driven inside. If the membrane is depolarised, that is the membrane potential is reduced from the resting potential, to the threshold of the sodium channels, approximately $-60$ mV. The sodium channels on the membrane open, allowing sodium ions to flow into the cell due to the chemical gradient, resulting in further depolarisation of the membrane. When the membrane potential reaches $+30$ mV, the sodium channels close and the potassium channels open. The chemical gradient of potassium overall ions results in potassium ions moving outside the membrane, repolarising the membrane back towards the resting potential. This process typically takes 1 ms. As part of the axon reaches $+30$ mV this depolarisation causes the sodium channels further down
Figure 1.1: Diagram of a spinal motor neuron, showing both the cell body and synapse to a muscle (Kandel et al., 2000), copyright McGraw-Hill Education.
1.1. Nervous System

Figure 1.2: Example of $K^+$ and $Na^+$ ion channels at resting potential. Figure from (Kandel et al., 2000), copyright McGraw-Hill Education.

Figure 1.3: Representation of action potential propagation from right to left. The active region (1) causes local current flow that causes depolarisation in the region ahead (1). Figure from (Kandel et al., 2000), copyright McGraw-Hill Education.
the axon to open, resulting in the activation and subsequent depolarisation to be transmitted along the axon. This moving depolarisation of the cell is known as an action potential. (Kandel et al., 2000)

Once the action potential reaches the end of the axon, it is transmitted to the dendrites of the next neuron, or to the target muscle cell, through a synapse. Most synapses use neurotransmitter chemicals, which are released by the presynaptic neuron and bind to receptors on the postsynaptic neuron’s membrane. Neurotransmitters can be excitatory or inhibitory, depending on the postsynaptic cell’s receptor. The postsynaptic cell’s response to the synaptic input can depend upon multiple signals from other neurons. This signal dependence can be both temporal (i.e. a minimum number of signals in a time period), or spatial (i.e. a minimum number of simultaneous signals triggering a region of a neuron). This processing of signal from different neurons forms the basis of information processing performed by the nervous system (Kandel et al., 2000).

Myelination of axons increases the rate of propagation of action potentials by allowing the local ion current loops to skip the part of the axon covered by the myelin. As a result, depolarisation at one node of Ranvier, jumps to the next node which may be 1 – 2 mm away. This increases the speed of propagation and reduces the energy required by the nerve. (Kandel et al., 2000)

1.1.2 Neural Stimulation

Neural stimulation is the application of an artificial stimulus to provoke a neural response (Navarro et al., 2005). Almost all neural tissue is excitable by the application of an electric field. As such, direct stimulation with electricity has been the gold standard technique to artificially stimulate neurons for over 100 years (Navarro et al., 2005), and is used both experimentally and clinically. The discovery of electrical stimulation has given rise to a broad field, allowing investigation into the anatomy and physiology of the nervous system and development of therapeutic tools and neural implants. The mechanism of electrical stimulation is straightforward. When an electric field is applied to a neuron, it can depolarise the cell membrane, provided that the field is greater than the depolarisation threshold. This depolarisation creates an action potential which is then propagated through the neural network. An example of the response seen from electrical stimulation is shown in Figure 1.4.

Electrical stimulation has allowed for development of both sensory devices, such as the cochlear implant (Clark, 2003) and bionic eye (Ong and da Cruz, 2012), neuromuscular prosthesis, to return control of the muscular system where the nerves transmitting information from the brain have been damaged (Peckham and Knutson,
Figure 1.4: Example of auditory brainstem response from electrical stimulation in the cochlea. Trace shows artefact from electrical stimulation, auditory system response and myogenic response. Note that the electrical artefact been attenuated by 100 times for presentation purposes.

2005) and treatment of neurological disorders such as epilepsy (Durand and Bikson, 2001; Sun et al., 2008).

Many other neural stimulation techniques exist, although they have not been as widely used as electrical stimulation. Transcranial magnetic stimulation (TMS) uses electromagnetic induction to produce localised electric fields in brain. It is non-invasive and can be used to simulate areas just a few millimetres across (Hallett, 2000; Wassermann and Lisanby, 2001). Nerves are also known to respond to direct mechanical stimulation: Norton (2003) demonstrated that ultrasound in the presence of a magnetic field could locally stimulate neurons.

Despite the widespread use of electrical stimulation in neural implants, it has a number of limitations. This includes the spread of current from the electrodes, leading to a lack of spatial resolution. As nerves and axons are tightly bundled together, stimulating over a region of just \( \sim 1 \) mm will trigger many neurons, not all of which may be desired. An example of this limitation is the cochlear implant and is discussed in Section 1.2.2. While electrodes can be made smaller to increase spatial resolution, there are limits on the charge that can be injected with smaller electrodes before irreversible electrochemical reactions occur which can damage the tissue (Shepherd et al., 2013).
1.2 The Cochlear Implant

An basic understanding of the physiology of the cochlea and functionality of the cochlear implant is important for this thesis to show the potential benefits of optical stimulation. Presented here is an overview of the anatomy and physiology of the cochlea (Section 1.2.1) and function of the cochlear implant (Section 1.2.2). For a full understanding, readers are referred to the texts by Kandel et al. (2000) and Clark (2003).

1.2.1 The Cochlea

Human hearing is a highly complex system which allows us to perceive a wide dynamic range and to pick out and follow individual voices in noisy environments. The ear is comprised of many parts as shown in Figure 1.5. It can be broken up into three main components: the outer ear, the middle ear and inner ear. The outer ear captures sounds from the environment and passes it though the external auditory canal to the eardrum (tympanic membrane, Figure 1.5). The vibrations on the eardrum are then amplified by the middle ear (tympanum Figure 1.5), an air filled cavity containing three small bones. In addition to amplification of the acoustic energy, it also impedance matches sound in air to water, maximising the transmission of acoustic energy to the inner ear (cochlea). Finally the sound reaches the inner ear, where the vibrational sound energy is converted into neural impulses that are interpreted by the brain. This conversion is performed by the organ of Corti, which has hair cells that respond to vibration and convert them to neural impulses. Dendrites of the spiral ganglion neurons synapse to the hair cells and take the neural signals to the auditory pathways in the brain (Clark, 2003).

The cochlea, or inner ear, is a bony chamber arranged in a spiral shape, similar to the shape of a snail’s shell. It is filled with perilymph, a fluid similar to cerebrospinal fluid. As shown in Figure 1.6, the internal chamber of the cochlea is divided into three ducts - the scala tympani, scala vestibuli and scala media - by the basilar membrane and Reissner’s membrane. The basilar membrane is a flexible membrane that vibrates as a travelling wave, according to the sound that is delivered by the outer and middle ear. When a clean single tone is delivered to the cochlea, the basilar membrane vibrates at a point along the length of the cochlea corresponding to that frequency: high frequencies vibrate at the base while low frequencies are more apical. When a complex sound is delivered, the basilar membrane vibrates in positions corresponding to the spectral composition of the sound. This observation of the spectral deconstruction by the basilar membrane was first observed by Geog
1.2. The Cochlear Implant

Figure 1.5: Figure showing the anatomy of the ear (Kandel et al., 2000), copyright McGraw-Hill Education.
von Békésy, under stroboscopic illumination (Manley et al., 2012; Von Békésy, 1960) and was key to understanding the tonotopic organisation of the cochlea. The auditory system is described as tonotopic (Talavage et al., 2004), simply means that neurons are organised so frequencies close to each other are represented by neurons close to each other. This tonotopic organisation is present in the cochlea and many other parts of the auditory system (Talavage et al., 2004). The organ of Corti, between scala tympani and scala media is the sense organ that converts these mechanical vibrations into neural impulses. Hair cells in the organ of Corti respond to this local vibration, stimulating the spiral ganglion neurons. Therefore, the tonotopic organisation in the cochlea results from the travelling wave present in the basilar membrane. High frequency response and neurons are found near the base and with corresponding lower frequencies towards the apex. This tonotopic organisation has enabled the success of cochlear implants, as it allows for relatively simple targeting of neurons corresponding to different frequencies (Clark, 2003; Kandel et al., 2000).

Role of hair cells in hearing

The transduction from mechanical vibrations into neural impulses is performed by the sense organ, known as the organ of Corti. This organ is comprised of mechanically sensitive hair cells, both inner and outer, along with a range of support cells. The inner hair cells are arranged in a single row, which runs along organ. While the outer hair cells are typically arranged in three rows, further out from the centre of the cochlea spiral. A cross section of the organ of Corti, showing the physical arrangement is shown in Figure 1.6C. Additionally the neural connections between the inner and outer hair cells and spiral ganglion neurons are shown in Figure 1.7. There is a big difference in the neural connections between the two types of hair cells. Approximately 90% of spiral ganglion neurons terminate on the inner hair cells. On average the inner hair cells connect to ten neurons and are the sole terminal for those neurons. There are additionally a number of efferent neural processes, primarily connected to the outer hair cells. In contrast to the afferent processes, efferent connections to the inner hair cells are sparse. This neural arrangement gives two important conclusions: the bulk of information delivered to the auditory system is from the inner hair cells and that the outer hair cells may provide some form of feedback from the auditory system due to the efferent neural connection.

Outer hair cells are embedded in the tectorial membrane and bend upon application of force from mechanical vibrations. This bending causes specific ion channels to open and close, modulating the receptor potential (Russell et al., 1986). Additionally, the outer hair cells contract or elongate in response to electrical stimulation (Santos-
1.2. THE COCHLEAR IMPLANT

Figure 1.6: Anatomy of the cochlea. A) The overall structure of the inner, including vestibular system and cochlear spiral showing how the fluid filled tubes spiral around. B) A cross section of the cochlear spiral showing the arrangement of the three fluid filled ducts and the location of the basilar membrane and the hearing sense organ, the organ of Corti. C) A cross section of the organ of Corti, showing the arrangement of the inner and outer hair cells. Figure adapted from (Kandel et al., 2000), copyright McGraw-Hill Education.
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Figure 1.7: Neural connections in the organ of Corti. The majority of afferent neurons connect to the inner hair cells, which connect to average of 10 axons. A few afferent neurons connect to the outer hair cells, usually with one neuron connected to many hair cells. There are a number of direct efferent connections to the outer hair cells, but very few to the inner. Figure from (Kandel et al., 2000), copyright McGraw-Hill Education.

Sacchi, 1989). This elongation and contraction acts to amplify the vibrations on the basiliar membrane and in a quiet environment human ears are capable of emitting sound (Hudspeth, 2005). Additionally, the mechanical properties of the outer hair cells are tuned to a specific frequency corresponding to the tonotopic mapping of the cochlea. This change in frequency sensitivity increases the spectral resolution in the cochlea, by narrowing the region that responds to a specific frequency (Kandel et al., 2000). Therefore, outer hair cells play an active role in amplifying the motion of the basilar membrane and enhancing the sensitivity of human hearing. This enhancement allows detection of vibrations as small as 0.3 nm (Narayan et al., 1998). This feedback system allows for the perception of the huge dynamic range that humans are capable of.

Deafening

When performing electrical stimulation experiments in animals without the neural loss typical of sensorineural hearing loss, it is important that the animals are acutely deafened to avoid contaminating the electrophysiological recordings with electophonic
activity (Shepherd and Javel, 1997; Hardie and Shepherd, 1999). As the lasers used for INS can generate an acoustic click from the laser pulse, which has been shown to stimulate hair cells in the cochlea (Teudt et al., 2011) it is important to ensure that an optoacoustic effect does not contaminate the response from laser light interacting with neurons.

Deafening can be performed through exposure to high intensity sound (Kiang et al., 1976) or by chemically deafening through use of with ototoxic aminoglycosides (Ylikoski et al., 1974; Kiang et al., 1976) such as neomycin (Leake and Hradek, 1988) or kanamycin (Wise et al., 2005). Ototoxic aminoglycosides deafen by disabling and killing the hair cells in the cochlea (Lim, 1986). They create an immediate and reversible blockage of transduction channels on the hair cells and kill hair cells by impairing cell maintenance machinery required for cell repair and survival (Lim, 1986). Immediate blockage of channels has been shown to act through a number of mechanisms, these include: a blockage of the mechanotransducer channels (Alharazneh et al., 2011; Ricci, 2002), blocking the outer hair cell ACh receptor channel (Blanchet et al., 2000) and by blocking the Ca\textsuperscript{2+} activated K\textsuperscript{+} channels (Dulon et al., 1995). Hair cell death appears to be due to reactive oxygen species (Rybak and Ramkumar, 2007; Hirose et al., 1999) and activation of caspase, a protease involved in cell death (Cunningham et al., 2002).

Care must be taken when deafening, as inadequate exposure to ototoxic chemicals can leave hearing in the apical region of the cochlea. The click threshold test has also been shown to not always be a good indicator of the extent of hearing loss (Shepherd and Martin, 1995). Furthermore, there is differential vulnerability between inner and outer hair cells and basal and apical cells to aminoglycosides (Lim, 1986, 1975; Halm et al., 2013). Basal outer hair cells are more susceptible to damage compared to apical outer hair cells from aminoglycosides due to increased susceptibility to free radical attack from reactive oxygen species (Sha et al., 2001) and differences in uptake of aminoglycosides (Dai and Steyger, 2008). Inner hair cells have been found to be more resistant than outer hair cells to aminoglycosides (Sha et al., 2001; Dallos and Harris, 1978; Ding et al., 2010). This basal-apical differential vulnerability is similar to that observed from hearing loss to noise exposure (Sha et al., 2001).

Neomycin is often added directly added to the cochlea (Hardie and Shepherd, 1999) and is typically aspirated through the cochlea to ensure the cochlea is left profoundly deaf from thorough ototoxin exposure (Hardie and Shepherd, 1999) or administered by intramuscular injections (Leake and Hradek, 1988). Direct addition of neomycin to the cochlea is used as an acute deafening procedure, to prevent electrophonics from affecting the recordings (Hardie and Shepherd, 1999; Landry
et al., 2011).

An animal model of human sensorineural hearing loss, with spiral ganglion neuron degeneration, can be produced by deafening animals and leaving them to allow spiral ganglion neuron degeneration to represent human loss of spiral ganglion neurons (Nadol et al., 1989; Hardie and Shepherd, 1999). Chronic deafening results in significant degradation in the spiral ganglion neurons and other auditory neurons (Hardie and Shepherd, 1999), in addition to damage to the organ of Corti. This neural damage can change the electrical thresholds required to generate a response in experiments targeting the spiral ganglion neurons, providing a representation of neuronal behaviour from hearing loss in humans (Webster and Webster, 1981). Chronic deafening can be performed with injections of neomycin (Leake and Hradek, 1988) or combined injections of kanamycin and frusemide, which has been shown to produce consistent symmetric bilateral deafness in animals (Gillespie et al., 2003; Shepherd et al., 2008; Landry et al., 2011).

**Temperature**

Fluctuations in temperature can affect the behaviour and response observed from the cochlea. Kahana et al. (1950) observed that changing the temperature at the round window of the cochlea could change the response observed for electrical stimulation. More recent work has shown that cooling the cochlea can raise the threshold to generate a CAPs (Ohlemiller and Siegel, 1994) but does not significantly change the temporal behaviour of responses (Ohlemiller and Siegel, 1998). These results indicate that care needs to be taken when performing experiments on the exposed cochlea, to prevent excessive cooling from occurring.

1.2.2 The Cochlear Implant

The cochlear implant is a highly successful bionic device (Clark, 2003) and is used by over 300,000 patients world wide. It is typically used for patients who are suffering hearing loss due to damage to the hair cells in the inner ear, which convert vibrational sound energy into neural impulses. The implant makes use of the tonotopic mapping in the cochlea to selectively stimulate neurons corresponding to different frequency bands, this allows for enough information to be delivered to provide speech perception.

A full description of the history and design of the cochlear implant is out of the scope of this thesis and readers are referred to a comprehensive text on the implant for more detail (Clark, 2003).
Current Status

Current cochlear implants use up to 22 electrodes, depending on the manufacturer. These electrodes are separated by 0.75 mm in the case of Cochlear Ltd’s implant, which uses 22 independent electrodes. However, due to current spread in the tissue and perilymph, the effective number of independent channels is often 4 – 8 (Friesen et al., 2001; Srinivasan et al., 2010), depending on the patient and resulting position of the implant in the cochlea. While this response is adequate to provide many patients with speech perception in a quiet area, most struggle with background noise (Clark, 2003).

Experiments where normal hearing patients have their sound delivery restricted to between 2 and 20 frequency bands, show that at least 20 channels are required for reliable speech perception in noisy environments (Friesen et al., 2001) and many cochlear implant patients do not significantly improve beyond the response seen with 8 channels. An example of this is shown in Figure 1.8 (from (Friesen et al., 2001)). Here normal hearing listeners are compared with cochlear implant listeners in recognition of HINT sentences as a function of discrete spectral channels or number of electrodes used. Despite each electrode theoretically being an independent spectral channel, cochlear implant listeners perform much less well than normal hearing listeners, especially with poor signal to noise. Additionally, music and melody recognition is thought to require at least 32 channels (Kong et al., 2004) and many current patients struggle with music perception (McDermott, 2004).

In a normal hearing cochlea, loudness is encoded by the firing rate of neurons. There is a 20 – 50 dB dynamic range from threshold to the point where neurons are saturated (Kiang et al., 1965). A dynamic range, greater than 50 dB, is encoded by neurons with different thresholds. Some neurons respond to low sound intensity, while other only respond at greater intensity. This combination of thresholds allows the full range of volumes perceived by normal hearing to be encoded (Liberman, 1978). This encoding limits the ability of current cochlear implants to encode loudness, as they are unable to discriminate between the nerves with a lower threshold and those with a higher threshold. Early implants had a limited range of approximately 4 dB, although current implants have a dynamic range of 10 – 20 dB (Zeng et al., 2002).

Much research has been directed towards reducing the current spread from electrodes to focus stimulation on a smaller groups of neurons (O’Leary et al., 2009). Techniques to localise the current include current steering through the use of multiple current sources and sinks such as tripolar stimulation (Srinivasan et al., 2010; Bonham and Litvak, 2008). However there is little evidence that these techniques reduce the spatial extent of neural excitation (O’Leary et al., 2009) and they have not shown
Figure 1.8: Recognition of HINT sentences as a function of spectral channels for normal hearing listeners and cochlear implant listeners, with different signal to noise levels. Despite theoretically having the same number of spectral channels, cochlear implant users do much worse than normal hearing listeners. Figure from (Friesen et al., 2001) is copyright AIP Publishing and is used with permission.
promise in clinical applications (Clark, 2003; Osberger and Fisher, 1999). Current focusing through the use of a phased array (van den Honert and Kelsall, 2007) is a similar technique that may overcome some of the limitations of current steering. However, it still requires higher current levels compared to traditional monopolar stimulation.

Other approaches to improve the response from current implants include the use of neurotrophic drugs to reduce spiral ganglion cell loss and to encourage the spiral ganglion neurons to grow new dendrites towards the implant (Wise et al., 2005; Gillespie and Shepherd, 2005). This outgrowth reduces the distance between the electrodes and target nerves, resulting in the potential for lower thresholds and greater spatial specificity (Gillespie and Shepherd, 2005). One challenge with this technique is ensuring nerves fibre remain organised (Wise et al., 2005), recently there has been some progress towards addressing this (Wise et al., 2010).

1.3 Optical Activation of Neurons

Despite the success of electrical stimulation for triggering neurons, there has been interest in improving electrical stimulation or developing alternative techniques without the disadvantages and limitation of electrical stimulation (Fenno et al., 2011; Richter et al., 2011a). The usefulness of many bionic implants has been limited by the spread of current from the electrodes and the lack of spatial specificity (Richter et al., 2011a). Similarly, neuroscience would benefit from an ability to turn individual neurons on or off (Fenno et al., 2011). There has long been interest in using light to influence the behaviour of neurons (d’Arsonval, 1891; Fork, 1971; Hirase et al., 2002) partially as it is less invasive than other techniques. Recently, a number of techniques have been developed to use light to trigger neurons (Boyden et al., 2005; Wells et al., 2005b).

Light has been known to influence the behaviour of neurons since the work of d’Arsonval (1891). While there are some neurons, such as photoreceptors in the eye, that have become specialised in order to respond to light, most neurons are not normally activated by exposure to light (Kandel et al., 2000). Fork (1971) showed that abdominal ganglion neurons in Aplysia californica respond to 488 nm laser light, through a reversible mechanism, despite the cells not being photosensitive. Building on the work of (Fork, 1971) and others, Balaban et al. (1992) found that He-Ne laser irradiation ($\lambda = 632.8$ nm) of subesophageal ganglia of Helix pomatia promoted membrane depolarisation and action potentials in spontaneously active neurons. Hirase et al. (2002) demonstrated that neurons could be triggered through
exposure to pulses of light from a femtosecond laser via a two photon mechanism. They found two regimes of activation: low intensity, long duration exposure produced a sustained depolarisation; while high intensity, short duration exposure could induce fast depolarisation. The two regimes appeared to be due to different mechanisms.

1.3.1 Optogenetics and Caged Molecules

Recently, a number of techniques to introduce light sensitivity to neurons have been developed. Early techniques, were able to increase the sensitivity of neurons and generate action potential spikes with laser exposure timescales of seconds (Zemelman et al., 2002, 2003). These techniques either relied upon genetically modifying the target neurons to introduce photosensitive receptors in the neurons (Zemelman et al., 2002), or the introduction of caged molecules that bind to specific ion channels and modify their behaviour when exposed to light (Zemelman et al., 2003; Kramer et al., 2005; Callaway and Katz, 1993).

A technique to introduce sensitivity to light exposure on the timescale of milliseconds was introduced by Boyden et al. (2005). In that work, neurons were transfected with an algal protein Channelrhodopsin-2 (ChR2), a rapidly-gated light-sensitive cation channel. This advance allowed precise control of neuronal firing on the order of milliseconds when exposed to blue light. This paper led to the development of the field of optogenetics, which describes the use of light to control neurons with tight spatial and temporal confinement (Zhang et al., 2007; Kramer et al., 2009). In addition to activating neurons, opsins which suppress neural activity have also been developed. This allows neurons to be activated and deactivated by exposing them to different colours of light. These techniques have been utilised to further our understanding of neural disorders (Tye and Deisseroth, 2012) and there is interest in using optogenetics to treat blindness and Parkinson’s disease (Kramer et al., 2009).

Despite the power of these techniques and their importance in the field of neuroscience, the potential for use in bionic devices or treatment of conditions in humans is currently limited. Optogenetic techniques require transfection of genes into neural cells or the introduction of caged molecules, which may have significant regulatory hurdles for human use (Sahel and Roska, 2013; Knüpfer and Boyden, 2012). Additionally, most opsins are limited to a maximum response rate of just 40 Hz. The maximum sustained rate of stimulation is important for bionics applications: for example, the current cochlear implant uses a stimulation rate of 900 Hz (Clark, 2003). However, new opsins are emerging which allow spike trains of up to 200 Hz (Gunaydin et al., 2010), so it is feasible that this limitation can be overcome by further research. Recently, optogenetic techniques have been applied to the cochlea.
to improve understanding of the neurophysiology and potentially develop improved implants (Darrow et al., 2013; Schwarz et al., 2013; Shimano et al., 2013).

1.3.2 Infrared Neural Stimulation

Infrared light has been demonstrated as an alternative technique for optical stimulation of nerves, without the need for genetic manipulation or other interventions (Richter et al., 2011a; Wells et al., 2005b). The technique of using infrared light to stimulation neurons has been coined infrared neural stimulation (INS). The use of infrared light has a number of potential advantages over electrical stimulation: finer spatial resolution can in principle be achieved, no direct contact between the stimulation source and target neurons is required, there is no electrochemical junction between the source and target tissue, and there is no stimulation artefact on the recording electrodes. Disadvantages of INS include: heating of the tissue to level that could cause damage (Wells et al., 2007a; Thompson et al., 2012) and a restriction on the maximum depth of stimulation due to absorption of light in the intervening tissue (Thompson et al., 2012). Compared to optogenetic and caged molecule techniques, INS requires no modification of the target tissue as it only relies upon the absorption of infrared light by water in the tissue (Richter et al., 2011a; Shapiro et al., 2012).

Pulses of mid-infrared light were first observed to elicit responses in mammalian nerves by Wells et al. (2005b). They exposed the sciatic nerve of rats to irradiation from a free electron laser (FEL), with wavelengths between 2 $\mu$m and 10 $\mu$m, observing compound nerve action potentials (CNAP) and compound muscle action potentials (CMAP) with a strong spatial specificity. Additionally a Ho:YAG laser ($\lambda = 2.12$ $\mu$m) produced a response in the sciatic nerve. Histological analysis of the nerves after stimulation showed no evidence of tissue damage, confirming that the energy deposited is below tissue damage thresholds. This work was expanded upon in (Wells et al., 2005a), where the dependence of the damage and stimulation thresholds on wavelength, and therefore absorption coefficient, were further investigated. Wavelengths with lower water absorption ($\mu_a \sim 2$ mm$^{-1}$), were found to have a greater safety ratio between the energy required for stimulation and the threshold for damage.

1.4 INS Applications

Since the initial demonstration of INS in the rat sciatic nerve by Wells et al. (2005b), the technique has been extended and demonstrated in a number of other models. A summary of these other targets is presented in Table 1.1, along with the laser
wavelength, pulse length, fibre diameter and resultant threshold found. Table 1.1 is not intended as a comprehensive summary of all INS studies, but rather to provide an overview of the main targets that have been investigated.

A summary of the radiant exposure and pulse duration for different optical stimulation techniques is shown in Figure 1.9. The figure includes: INS in peripheral nerves, INS in the cochlea, optogenetic techniques and opto-acoustic stimulation of the cochlea. Cross markers show the individual thresholds from different papers, while the coloured ovals indicate a rough idea of the overall radiant exposure to pulse length relationship. It clearly shows that INS in the cochlea operates in a different regime to INS with other neural targets. The optoacoustic markers show results from (Wenzel et al., 2009; Schultz et al., 2012), where sound from the absorption of laser light generates an acoustic click. It should also be noted that pulses similar to those used in INS also create audible sounds upon absorption in water (Teudt et al., 2011).

1.4.1 INS in peripheral nerves

Work in the rat sciatic nerve has been extended to examine both the safe range of stimulation parameters (Wells et al., 2007c) and a comparison between optical and electrical stimulation modalities (Wells et al., 2007b). Studies on the safety margins (Wells et al., 2007c) found that that the threshold to stimulate tissue and
### Table 1.1: Summary of the various experimental parameters for a range of different INS studies in the literature. This table does not present data from every study discussed in this section, but provides an overview of the main results.

<table>
<thead>
<tr>
<th>Author</th>
<th>Neural Target</th>
<th>Wavelength (nm)</th>
<th>Stimulation Threshold (mJ.cm⁻²)</th>
<th>Pulse Length (ms)</th>
<th>Fibre Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wells et al. (2005b)</td>
<td>Rat sciatic nerve</td>
<td>2120, 2000 – 6100¹</td>
<td>320</td>
<td>0.25</td>
<td>600</td>
</tr>
<tr>
<td>Wells et al. (2007c)</td>
<td>Rat sciatic nerve</td>
<td>2120</td>
<td>340</td>
<td>0.35</td>
<td>600</td>
</tr>
<tr>
<td>Wells et al. (2007b)</td>
<td>Rat sciatic nerve</td>
<td>2120</td>
<td>320</td>
<td>0.35</td>
<td>600</td>
</tr>
<tr>
<td>Teudt et al. (2007)</td>
<td>Gerbil facial nerve</td>
<td>2120</td>
<td>710</td>
<td>0.25</td>
<td>600</td>
</tr>
<tr>
<td>Fried et al. (2008b)</td>
<td>Rat cavernous nerve</td>
<td>1870</td>
<td>1000</td>
<td>2.5</td>
<td>300</td>
</tr>
<tr>
<td>Fried et al. (2008a)</td>
<td>Rat cavernous nerve</td>
<td>1850 – 1880</td>
<td>350</td>
<td>2.5</td>
<td>400</td>
</tr>
<tr>
<td>Jenkins et al. (2010)</td>
<td>Embryonic quail heart</td>
<td>1875</td>
<td>810</td>
<td>2</td>
<td>400</td>
</tr>
<tr>
<td>Jenkins et al. (2013)</td>
<td>Adult rabbit heart</td>
<td>1851</td>
<td>7000</td>
<td>2.5 – 12</td>
<td>400</td>
</tr>
<tr>
<td>Cayce et al. (2011)</td>
<td>Rat somatosensory cortex</td>
<td>1875</td>
<td>140²</td>
<td>0.25</td>
<td>400</td>
</tr>
<tr>
<td>Izzo et al. (2006)</td>
<td>Gerbil cochlea</td>
<td>2120</td>
<td>18</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td>Izzo et al. (2007c)</td>
<td>Gerbil cochlea</td>
<td>1844 – 1873</td>
<td>6</td>
<td>0.035 – 1</td>
<td>200</td>
</tr>
<tr>
<td>Izzo et al. (2008a)</td>
<td>Gerbil cochlea</td>
<td>1923 – 1937</td>
<td>1.6</td>
<td>0.05 – 0.3</td>
<td>200</td>
</tr>
<tr>
<td>Richter et al. (2008)</td>
<td>Gerbil cochlea</td>
<td>1844 – 1873</td>
<td>3³</td>
<td>0.03 – 1.6</td>
<td>200</td>
</tr>
<tr>
<td>Duke et al. (2009)</td>
<td>Rat sciatic nerve</td>
<td>1875</td>
<td>1690⁴</td>
<td>2</td>
<td>400</td>
</tr>
<tr>
<td>Duke et al. (2012a)</td>
<td>Aplysia buccal nerve</td>
<td>1875</td>
<td>8930</td>
<td>2 – 3</td>
<td>100</td>
</tr>
</tbody>
</table>

¹At discrete wavelengths of 2.1, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6.1 µm
²Threshold not well defined, a change to intrinsic signal was observed at 0.14 J.cm⁻²
³Chronically deafened animals displayed greatly increased thresholds (over 10 fold), especially at longer pulse lengths
⁴Increased threshold compared to work by Wells et al. is due to smaller core diameter. Threshold reduced three-fold upon application of simultaneous electrical pulse at 95% threshold.
corresponding 95% confidence ranges did not overlap with the radiant exposures found to cause damage, when stimulation was performed at rates between 2 – 8 Hz. Damage was assessed by analysing histological samples for thermal lesions. Damage thresholds were found to be 0.7 J.cm\(^{-2}\) for a 1% probability of damage and 0.91 J.cm\(^{-2}\) for a 50% probability, when using histological analysis of exposed tissue. Additionally, higher pulse rates (5 – 8 Hz) were more likely to cause damage than 2 Hz. When optical and electrical stimulation modalities were compared, Wells et al. (2007b) found a near linear relationship between the radiant exposure and the measured compound nerve action potential (CNAP) response, similar to that observed when using electrical stimulation. Unlike the electrical response, the minimum CNAP response from an optical stimulus was 4 times smaller. This lower minimum response suggests greater spatial localisation with optical stimulation.

The suitability of other wavelengths of infrared light for stimulating the rat sciatic nerve was performed by McCaughey et al. (2010). In that work, wavelengths of 1450 nm, 1495 nm, 1540 nm and the more conventional 2100 nm were examined, using various diode laser sources. Stimulation was achieved with all laser sources, however it was most reliable when using the 1495 nm source. However, meaningful comparisons between the different wavelengths and laser sources is made difficult by the large variation between fibre diameters, beam divergences and pulse durations. Moreover, the long pulses (≥ 100 ms) used by the 1450 nm and 1540 nm sources are near the thermal diffusion time for the areas irradiated.

The gerbil facial nerve was stimulated using INS by Teudt et al. (2007). The authors used a Ho:YAG laser (\(\lambda = 2.12\) µm, \(\varnothing_{\text{core}} = 600\) µm, \(t_{\text{pulse}} = 250\) µs, \(f = 2\) Hz). Response to the irradiation was observed when using radiant exposures from 0.71 J.cm\(^{-2}\) to 1.77 J.cm\(^{-2}\), with amplitudes similar to that observed when using electrical stimulation. Histological analysis of higher radiant exposures revealed that damage was present at levels greater than 2.00 J.cm\(^{-2}\). In addition to measuring the response of nerves, the authors measured the profile of the beam resulting from transmission in air, Ringer’s lactate and muscle tissue. No change in the beam profile was observed when transmitted through ringer’s lactate when compared to air. However, transmission through tissue was found to broaden the spot when compared to the other media. These results showed that Beer’s law is a good first approximation for the spatial behaviour of light during INS, but scattering also plays a role in the propagation of light. The results suggested that INS could be beneficial clinically as a monitor of the facial nerve during surgery, as no contact to the nerve is required and it allows for greater spatial selectivity.

The rat cavernous nerve was stimulated using INS by Fried et al. (2008b) to estab-
lish the potential of INS for nerve mapping during nerve-sparing radical prostatectomy. A thulium fibre laser ($\lambda = 1870$ nm, $\varnothing_{\text{core}} = 300$ $\mu$m, $t_{\text{pulse}} = 2.5$ ms, $f = 10$ Hz, radiant exposure = 1.00 J.cm$^{-2}$) was used. The intracavernosal pressure was monitored and showed a similar response to the laser stimulation as occurred with conventional electrical stimulation. This result demonstrated the feasibility of INS for noncontact stimulation of the cavernous nerves. The technique was further optimised in (Fried et al., 2008a), where a tuneable laser ($1850$ nm $> \lambda > 1880$ nm, $\varnothing_{\text{core}} = 400$ $\mu$m, repetition rate = 10 Hz) was used. Optimal parameters of $1860$ nm $< \lambda < 1870$ nm with a minimum radiant exposure of 0.35 J.cm$^{-2}$ were found. The authors conclude that while promising, translating the technique from the rat model to human patients may be difficult as the target nerves are harder to visually identify in humans.

Jenkins et al. (2010) demonstrated that the heart of an embryonic quail could be paced using INS as a non-invasive technique. 1875 nm laser light was used and no damage was observed in the tissue. Optical pacing of the heart was further explored in (Jenkins et al., 2013). Here, pulses of 1851 nm light were used to pace adult rabbit hearts at radiant exposures of $6 - 11.8$ J.cm$^{-2}$ and pulse durations of 2.5 ms to 12 ms. Unlike other results for INS, a pulse duration of 8 ms was found to have the lowest radiant exposure stimulation threshold. Additionally, higher stimulation frequencies resulted in a lowering of optical thresholds, similar to that observed by Duke et al. (2012b). Using propidium iodine staining to determine damage, radiant exposures of 7.9 J.cm$^{-2}$ or above were found to cause some disruption of cell membranes, which may limit the duration over which this technique can be used.

An attempt to stimulate nerves $ex\ vivo$ was made by Cargill et al. (2008). They used a diode laser ($\lambda = 1.85$ $\mu$m, $P_{\text{peak}} = 5$ W, $\tau_{\text{pulse}} = 1 - 5$ ms, $\varnothing_{\text{core}} = 600$ $\mu$m). Ten nerve samples were extracted from mice and responded to electrical stimulation. However, no activation was observed when using optical stimulation. The authors speculated that the explanation for this discrepancy between $ex\ vivo$ and $in\ vivo$ may be due to the nerves being at room temperature rather than body temperature, or due to other differences between the mouse and rat animal models. Follow up discussion with one of the authors revealed that they were unable to perform further research as their laser broke$^5$.

1.4.2 INS in the Central Nervous System

The efficacy of INS to stimulate the brain was shown by Cayce et al. (2011). Here a diode laser ($\lambda = 1.875$ $\mu$m, $\varnothing_{\text{core}} = 400$ $\mu$m) was used to stimulate the somatosensory cortex of anaesthetised rats with pulse lengths of 250 $\mu$s at a repetition rate of

$^5$Private discussion with S. Jacques at Photonics West 2013
50 – 200 Hz and radiant exposure between 0.01 and 0.55 J.cm$^{-2}$. The intrinsic signal magnitude was monitored and found to display similar trends to those observed in (Cayce et al., 2010). Overall, INS was safely demonstrated in the CNS and maintained strong spatial isolation.

Stimulation of the macaque monkey primary visual cortex was performed by Cayce et al. (2014). An INS laser with wavelength of 1875 nm and fibre core diameter of 100, 200 and 400 µm were used. INS was able to produce enhancement of the response in the visual cortex to a visual stimuli when using a fibre diameter of 100 or 200 µm. When using the larger core diameter of 400 µm an inhibitory response was observed.

1.4.3 INS in the Cochlea

Electrical stimulation of the cochlea has produced one of the world’s most successful bionic devices (Clark, 2003). One current limitation of the implant is the spread of electrical current through the tissue and the perilymph, reducing the spatial selectivity that can be achieved with this stimulation modality. The improved spatial selectivity of INS potentially makes it a very attractive technique for stimulation of nerves in the cochlea.

INS of the cochlea was first performed in gerbils by Izzo et al. (2006) at Northwestern University. Optical radiation from a Ho:YAG laser ($\lambda = 2.12$ nm) targeting the modiolus was delivered to the cochlea by a 100 µm core diameter fibre, with pulse durations of 250 µs at 2 Hz and a distance of 0 – 500 µm from the target. Compound action potentials (CAPs) were observed in response to the laser pulses in both normal hearing and deafened animals in which the acoustic threshold had increased by approximately 40 dB. As a result of the deafening, the authors suggest that the interaction occurs directly with the nerves and is not mediated by an optoacoustic effect involving the hair cells. No evidence of neural damage was observed during the stimulation, suggesting that stimulation at this rate was safe for the duration of the experiment (6 hrs). Since this initial demonstration of INS in the cochlea, the Richter group at Northwestern University has been at the forefront of research in this field. Unless stated, the results discussed in this Section are from the Northwestern group.

While the work of Izzo et al. demonstrated that the auditory system responded to the optical stimulus, it did not demonstrate the proposed advantage of improved spatial selectivity, or limiting stimulation of the auditory system to a smaller frequency range than is possible with electrical stimulation. Spatial selectivity has been investigated with a range of techniques, including: fluorescent staining with c-FOS
to identify excited neurons; inferior colliculus (ICC) recordings which identify the
development of signals to different neural pathways and acoustic tone masking. Izzo
et al. (2007b) investigated the spread of excitation in the gerbil cochlea, using c-FOS
staining to identify neurons activated by optical, electrical and auditory stimuli. They
found that optical stimulation of neurons provided higher spatial selectivity
than electrical stimulation, with only neurons in the beam path activated, whereas
electrical stimulation led to a much greater spatial spread of excitation. Further work
on spatial selectivity was presented in Richter et al. (2011b) using recordings made in
the inferior colliculus (ICC). The inferior colliculus has tonotopic organisation which
correlates to the tonotopic mapping in the cochlea (Clark, 2003). This tonotopic
organisation therefore allowed recording from a multichannel electrode array to
measure the neural response in different regions of the cochlea and to examine the
spatial localisation of stimulation. Comparisons between recordings of acoustic tone
pips and INS stimulation were made in the ICC and revealed similar localisation.
This result suggests that an implant based upon INS could present an increased
number of frequency channels to a cochlear implant user. Further evidence of the
spatial selectivity of INS in the cochlea was presented by Matic et al. (2011), where
acoustic masking techniques were used to determine the frequency specificity of INS
stimulation. These results suggested that the spatial localisation of INS is similar
to that observed with acoustic stimulation, confirming the results of Richter et al.
(2011b).

A range of laser wavelengths with different absorption characteristics have been
used for INS (Richter et al., 2011a). The effects of different laser parameters have
also been investigated in the cochlea, including wavelengths of 1850 – 1870 nm (Izzo
et al., 2007c), 2120 nm (Izzo et al., 2006) and 1937 nm (Izzo et al., 2008a). The range
from about 1850 – 1870 nm is convenient for study, as there is a two-fold change
in the absorption coefficient for water ($\mu_a$) over the 20 nm change in wavelength.
This change in wavelength, with the corresponding change in water absorption
can be made by adjusting the temperature of the laser diode, thus allowing direct
comparison over this range, without any confounding factors. This change in the
water absorption coefficient was investigated by Izzo et al. (2007c) who stimulated
the gerbil cochlea with a diode laser in the wavelength range of 1.844 – 1.873 $\mu$m.
The laser light was delivered by an optical fibre located outside the round window,
giving a distance of $\sim$ 500 $\mu$m between fibre and target neurons. INS was most
effective at the less strongly absorbed wavelength of 1.844 nm ($\mu_a \approx 0.89$ mm$^{-1}$)
compared to 1873 nm which was more strongly absorbed ($\mu_a \approx 2.1$ mm$^{-1}$). This
investigation was extended in Izzo et al. (2008a), where a wavelength range of 1923
Chapter 1. Introduction

- 1937 nm ($\mu_a \approx 10.3 - 11.5 \text{ mm}^{-1}$) was used, with the fibre positioned as close as possible to the modiolus. Once again, a reduction in the CAP amplitude was observed when the wavelength was adjusted to the more strongly absorbed part of the range. These results show the importance of optimising the wavelength for the separation between emitter and target structure. Although the wavelength of light used by (Izzo et al., 2008a) was much more strongly absorbing, the distance between fibre and target was greatly reduced compared to (Izzo et al., 2007c).

A range of laser parameters have been used for INS in the cochlea and a number of studies have been performed on the effect of varying the pulse length used to stimulate the neurons. This was first investigated by Izzo et al. (2007c) where the diode laser allowed for pulse lengths of different durations to be used, unlike the Ho:YAG laser previously used (Izzo et al., 2006). A range of pulse lengths, between 35 µs and 1000 µs, were investigated. All of the pulse lengths were able to elicit a response, with the required energy per pulse scaling with the pulse length (i.e. displaying a power dependent response). However, the shortest duration pulses (35 µs) only required the same total energy as the 100 µs pulses, but a higher peak power than pulses 100 µs or longer. A further examination of the energy dependence on pulse length was performed by Izzo et al. (2008a), where pulses below 100 µs were found to require similar energy, but longer pulses required greater energy. This trend was also found in Richter et al. (2008), however, the data is more conflicted due to the addition of both acutely and chronically deafened animals.

As the rapid heating caused by INS laser pulses can generate an acoustic click (Teudt et al., 2011), any results of INS in the cochlea require controls with deafened animals. A number of studies have demonstrated that a response can be generated when the cochlea has been acutely deafened (Izzo et al., 2006; Richter et al., 2008; Kadakia et al., 2013). Deafening techniques commonly used involve applying neomycin directly to the round window or cochleostomy and allowing it to perfuse through the cochlea. This technique typically increases acoustic thresholds by at least 40 dB (Richter et al., 2008; Kadakia et al., 2013). Generally, these results have shown a small change in response between the normal hearing and acutely deafened cases. Chronically deafened animals have shown an increase in the energy required to generate a response (Richter et al., 2008), and with a sufficiently high concentration of neomycin no response to optical or electrical stimulation has been observed in some animals (Richter et al., 2008). The increased threshold in chronically deafened animals was attributed to a reduction in the spiral ganglion neuron count (SGNs) (Richter et al., 2008), which would imply that a larger volume of neural tissue must be stimulated to generate the same response. Recently, further research has been
performed using tone masking, to test whether there is any acoustic response to the laser. Results have suggested that the laser interaction with the target neurons is not primarily acoustic (Kadakia et al., 2013).

The target structures of INS were investigated in Moreno et al. (2011), to provide further detail on how the spatial selectivity and orientation of the fibre affects the stimulation result. A guinea pig cochlea was stimulated with 1862 nm laser light, after which, the fibre was replaced by a 250 µm hollow core fibre to deliver 10.6 µm radiation from a CO₂ laser. This light was used to ablate the tissue previously targeted by INS, leaving permanent markings for detection in a 3D microCT reconstruction of the cochlea. Comparisons between the microCT reconstructions, histology and physiological data showed a good match between physiological data and histology or microCT in determining which part of the cochlea was stimulated. The work also demonstrated the importance of the orientation of the fibre during INS.

As INS is mediated by heating of tissue, it is possible that high pulse repetition rates may damage the target tissue (Wells et al., 2005a). Maximising the usefulness of INS for implants may require high stimulation rates, on the order of 250 Hz to 1000 Hz, to be comparable to electrical stimulation in cochlear implants (Clark, 2003). Additionally, chronic safety studies will need to be performed to establish safety and damage thresholds over longer time periods. A number of papers have established that stimulation at low rates (10 – 20 Hz) does not cause any detectable damage for periods of 5 – 6 hours (Izzo et al., 2006, 2007c). Recently, reports of higher stimulation rates have been published. Rajguru et al. (2010) demonstrated stimulation rates of up to 250 Hz in a cat cochlea, with an 1860 nm laser and radiant exposure of $3.2 - 41.3 \text{ mJ.cm}^{-2}$ for durations of up to 10 hours. CAP values were stable during stimulation and no evidence of damage was observed in histological analysis of the cochlea post-stimulation. Similarly, Goyal et al. (2012) found no damage, either histological or physiological, when stimulating at 250 Hz for periods of up to 5 hrs ($\lambda = 1869 \text{ nm, } E_{\text{pulse}} = 25 \mu \text{J}$). A report on chronic stimulation in the cat cochlea was published by Matic et al. (2013). Normal hearing cats were implanted with an optical fibre targeting the spiral ganglion neurons in the cochlea, with the fibre coupled to a battery powered, backpack mounted laser at rates of up to 200 Hz ($\lambda = 1850 \text{ nm, } E_{\text{pulse}} = 12 \mu \text{J}$). The animals were stimulated for up to 8 hours a day for 30 days. No evidence of damage was observed, as post stimulation acoustic auditory brainstem response (aABRs) thresholds remained constant during the experiment. Additionally, no histological evidence of spiral ganglion neuron damage or thermal damage was found.

Currently, only limited behavioural studies have been performed on animals.
Matic et al. (2013) chronically implanted cats with an optical fibre targeting the spiral ganglion neurons in the cochlea. During stimulation, behavioural observations were made. Cats were individually released and observed while the laser was actively stimulating and while it was off: during stimulation the cats made more turns towards the implanted ear, suggesting perception of the laser pulses. However, proof of perception requires further experimentation to explicitly show that the animals perceive a sound.

Stimulation of the cochlear nucleus was demonstrated by Lee et al. (2009), from the Harvard Medical School. They recorded optically evoked auditory brainstem responses from a 400 µm core diameter fibre directed towards the cochlear nucleus. Unlike the results from the Northwestern group, this work targeted the central auditory neurons, rather than the more peripheral spiral ganglion neurons. They noted that the ABR recording was typical of that produced by acoustic stimulation and had a latency of 3 to 8 ms longer than that evoked by electrical stimulation in the same region. A follow up study found that after cutting the auditory nerve to deafen the animal, no response could be found from INS, while typical responses were found from electrical stimulation Verma et al. (2014).

A hair cell mediated response to laser stimulation, or optoacoustic stimulation, has been demonstrated by Wenzel et al. (2009), based in Hannover, Germany. In this work, green 532 nm laser light was delivered to the cochlea, generating an optoacoustic response. The laser pulses were 10 ns in duration with energies up to 23 µJ delivered by a 50 µm core diameter fibre. The response in most animals saturated at approximately 15 µJ and the response was similar when stimulation was performed with the fibre inserted through the round window, or with the round window intact. No damage was observed with stimulation at $E_{pulse} = 13$ µJ and a stimulation rate of 10 Hz for a duration of 30 mins. After deafening with kanamycin and ethacrynic acid, no response to the laser stimulation could be observed. These results suggest that the cochlea can respond to laser-induced acoustic events. As shown in Figure 1.9, the response to optoacoustic stimulation sits in a different pulse length and radiant exposure regime to INS in the cochlea.

In other research from Medical University Hannover, Schultz et al. (2012) showed that a range of wavelengths, from 400 nm to 2000 nm, can stimulate the cochlea with a nanosecond laser. In this regime, the response has a correlation between the water absorption or haemoglobin absorption of the wavelength. When the cochlea was deafened with neomycin no response to the laser was observed. While this suggests that INS could be mediated through an optoacoustic mechanism, results from (Richter et al., 2008) (Izzo et al., 2006) and (Kadakia et al., 2013) suggest that
the response seen during INS is not dependant on functioning hair cells.

1.4.4 INS in vitro

Cayce et al. (2010) stimulated thalamocortical brain slices or rats using a free electron laser ($\lambda = 2.51, 3.65, 4.00, 4.40, \text{ and } 5.30 \mu m, \tau_{\text{pulse}} = 5 \mu s, f = 6 - 30 \text{ Hz}$). More strongly absorbed wavelengths were found to require significantly lower thresholds to evoke a neural response than more weakly absorbed wavelengths. Similarly, higher repetition rates required less energy per pulse to be delivered to achieve stimulation. Additionally, larger spot sizes were found to require a smaller radiant exposure to evoke a response, as stimulating a larger area requires fewer neurons to react to the stimulus per unit area, compared to a smaller spot size. Lower energy levels were required to stimulate the tissue when compared to INS in the PNS, possibly due to the increase in excitable tissue per unit area.

A number of recent studies have used whole cell patch clamping techniques to investigate the mechanisms of INS (Brown et al., 2013). These studies typically use laser wavelengths in the 1850 – 1890 nm range with 1 – 15 ms duration pulses (Shapiro et al., 2012; Albert et al., 2012). A full discussion of these papers is found in Section 1.4.8.

1.4.5 Light delivery techniques

Optical fibres have provided the standard delivery mechanism for many INS experiments, as they are well-established optical components and relatively easy to use. A variety of standard silica core fibres have been used with a range of diameters, including for example, 600 $\mu m$ (Wells et al., 2005a, 2007a), 400 $\mu m$ (Duke et al., 2012b; Shapiro et al., 2012), 200 $\mu m$ (Richter et al., 2008) and 100 $\mu m$ (Albert et al., 2012; Izzo et al., 2006). Optical fibres are waveguides that trap light in the core of the fibre by total internal reflection (Snyder and Love, 1983). Optical fibres have a core and cladding, most commonly constructed of silica. The refractive index of the core is slightly higher than that of the cladding, so that the light is reflected if the angle of incidence is above the critical angle and cannot be refracted. Therefore, light is only captured or coupled into the fibre if its angle of incidence with the fibre is less than the acceptance angle of the fibre. This acceptance cone is generally described by the numerical aperture (NA). A large numerical aperture has a wider acceptance cone. Fibres are typically protected from the environment with a number of jacket or buffer layers. For typical INS experiments, these protective layers are removed near the emitting end of the fibre. Further details on optical fibres are provided...
in Chapter 2.2.1 and readers are referred to the comprehensive text on waveguides (Snyder and Love, 1983).

While fibres have been able to deliver light to a strongly localised area and are able to display a significant improvement over electrical stimulation for some applications (Richter et al., 2011b), there is interest in alternative delivery techniques that are able to deliver light closer to the nerves or to multiple nerves from a single source for both INS and other optically-mediated nerve stimulation techniques (Chernov et al., 2012).

An implantable multiwaveguide device has been the topic of some research. Abaya et al. (2012a) developed a Utah slanted optrode array (USOA), similar to the Utah slanted electrode array, which has allowed for more specific electrical stimulation of neurons in nerve trunks than was achievable with traditional electrodes (Normann et al., 2012). The USOA is aimed at delivering light to deeper tissue and to allow an even more selective stimulation of nerve trunks. The USOA is micromachined from a silicon wafer and etched to form narrow shanks 0.5 mm to 1.5 mm in length. Transmission efficiency varied between 2 - 10% depending upon the diameter of the fibre used to couple light into the array. Losses in coupling are due to Fresnel reflection between the different interfaces, which are increased by the high refractive index of silicon compared to glass, and losses due to the tapering of the tip. The Utah slanted optrode array was improved upon in (Abaya et al., 2012b), which reported a 3D silica optrode array with non-tapering tips. This resulted in a large increase in fibre-to-nerve coupling efficiency of up to 70% and significantly reduced transmission losses in the shank.

A laparoscopic probe designed to deliver a collimated top hat beam profile to allow uniform illumination of the cavernous nerves was developed in (Tozburun et al., 2010). The probe delivered a beam with a diameter of 1 mm, when coupled to a thulium fiber laser ($\lambda = 1870$ nm). This probe showed similar results to those from previous work using a fibre (Fried et al., 2008b) and may allow easier targeting of nerves for INS.

Optogenetics has also generated interest in advanced light delivery techniques. Typically optogenetics requires a lower laser power level (see Figure 1.9) and therefore can accept reduced coupling efficiency and greater losses. Zorzos et al. (2010, 2012) reported custom fabricated waveguides, capable of delivering light in a 3D pattern in tissue. Holographic patterning of light onto neural tissue was demonstrated by (Farah et al., 2013; Reutsky-Gefen et al., 2013). This technique uses a spatial light modulator to deliver light to individual neurons that have been photo-sensitised with optogenetic treatments or by using photo-absorbers (discussed in Section 1.4.7)
1.4.6 Combined optical and electrical stimulation

One of the disadvantages of INS is the heat deposited in the tissue, especially at high repetition rates (Izzo et al., 2008b; Thompson et al., 2013c). Any reduction in the total energy delivered to the tissue would be advantageous for the development of implants. Additionally, the current laser sources consume significantly more power than existing electrical implants such as the cochlear implant. Minimising total energy consumption will be important in developing portable and compact implantable stimulation systems. Duke et al. (2009) proposed using sub-threshold electrical depolarisation to reduce the optical energy required for stimulation. An initial investigation (Duke et al., 2009) found a three fold reduction in the optical energy when the electrical stimulus was 90% of threshold. These results showed the potential of electrical-optical hybrid stimulation. The strongest response was observed when the two pulses ended simultaneously. Additionally, the authors found that the relationship between increasing electrical current and required optical energy did not follow a linear relationship, implying that the two stimulation modalities do not function by the same mechanism.

Electrical-optical hybrid stimulation was further investigated in (Duke et al., 2012a). Here a rat sciatic nerve and Aplysia californica buccal nerve were stimulated using either a 2.12 \( \mu \text{m} \) Ho:YAG laser or a 1.875 \( \mu \text{m} \) diode laser and standard electrodes. For the Aplysia experiments, the light was coupled into a 100 \( \mu \text{m} \) or 200 \( \mu \text{m} \) core diameter fibre to match the nerve size; while for the rat sciatic nerve, the light was delivered by a 400 \( \mu \text{m} \) or 600 \( \mu \text{m} \) core diameter fibre as the nerve trunk is larger in this model. Both the temporal and spatial parameters of the optical and electrical stimuli were investigated. The authors found that there was a strong spatial dependence on the location of the optical stimulus relative to the electrodes, that excitation could only be performed when stimulated near the cathode and that the excitable area was larger when the electrodes were configured transverse to the nerve than parallel. Additionally, in Aplysia, increasing the optical radiant exposure could cause inhibition of the nerve, even when the electrical pulse was set to 110% of the threshold. The authors also noted that the choice of laser source greatly affected the performance of hybrid stimulation: although both wavelengths had the same absorption coefficient in water, the Ho:YAG showed greater reproducibility than the diode.

The muscular response due to exposure of neurons to electrical-optical hybrid stimulation was investigated in (Duke et al., 2012b). Here a rat sciatic nerve was exposed to electrical and hybrid electrical-optical stimulation and the force generated in the plantarflexor muscles was measured in response to the different stimulation
modalities and parameters. The optical stimulus was delivered by a 400 µm core diameter optical fibre connected to a diode laser (λ = 1875 nm), the light was delivered through a Sylgard nerve cuff which was found to have 93% transmission at the wavelength used. The nerve was stimulated at rates of 15 and 20 Hz for a period of 1 second. Unlike electrical stimulation, responses to hybrid stimulation increased during a pulse train reaching a plateau by the 20th pulse. Additionally, an isolated optical stimulus before the hybrid pulse trains showed an increase in force generated. These results suggest that an increase in the baseline temperature of the nerves is a contributing factor to the response observed during hybrid stimulation.

Hybrid electrical-optical stimulation has been used to show inhibition of electrical responses in vivo (Duke et al., 2013) and spontaneous neural activity in vitro (Liljemalm et al., 2013). Use of a pulsed INS laser reversibly inhibited action potential creation and blocked action potential conduction in both rat sciatic nerve and Aplysia buccal nerve (Duke et al., 2013). Infrared neural suppression is likely due to thermal block, which has previously been observed (Davis et al., 1976). However, the technique presented allowed for suppression with a spatial resolution as small as 100 µm and temporal resolution on the order of \( \sim 500 \mu s \). Use of this technique may allow non-invasive investigation of neurological tissue and could potentially be applied to treating neurological disorders.

Overall, the work on electrical-optical hybrid stimulation suggests that the optical energy requirements can be reduced by up to 90%, without a reduction in spatial localisation compared to INS alone. However, this reduction is dependent upon electrode position relative to the target neurons.

**1.4.7 Nanoparticle enhanced INS**

An alternative technique to enhance INS is by adding light absorbing materials to the tissue. Farah et al. (2013) introduced micron scale photo-absorbing particles (iron oxide) to cultured rat cortical cells. The light was holographically patterned onto the target cells, reducing the total exposure of light to the culture. Using the combination of photo-absorbers and patterned light, significant reductions in the energy required to achieve stimulation were observed. Additionally, this technique could allow for the use of wavelengths that are not strongly absorbed by water, thus allowing greater penetration depths to be obtained.

The use of gold nanorods in cells to absorb near infrared light was demonstrated by Paviolo et al. (2013). Here cells cultured with gold nanorods were found to produce intracellular Ca\(^{2+}\) transients in response to laser light at the plasmon resonance wavelength of the gold nanorods. As INS also produces intracellular Ca\(^{2+}\) transients
(Dittami et al., 2011), the authors speculated that absorbing nanoparticles could be used to improve the efficiency of INS.

1.4.8 Mechanisms behind INS

The mechanisms behind INS have been the topic of some discussion in the literature and a number of potential mechanisms have been identified which may contribute to the response observed from laser exposure.

Mechanisms of INS were first investigated by (Wells et al., 2007a), using a Ho:YAG laser ($\lambda = 2.12 \mu m$), free electron laser ($\lambda = 2.1 \mu m$) and diode laser ($\lambda = 1.87 \mu m$) to stimulate the rat sciatic nerve. They found that a photothermal interaction due to water absorption was the most likely mechanism behind INS, rather than photomechanical pressure waves or photochemical mechanisms. As the pulses used are well below stress confinement there is not a large pressure wave generated, reducing the possibility of a photomechanical mechanism. Photochemical effects were ruled out as direct photochemistry requires a photon energy greater than that found at the wavelength used for INS ($< 0.1$ eV). Additionally, transient tissue heating or a thermal gradient with respect to time was found to be required in order to achieve neural activation, as simply heating the tissue does not generate a response. The authors speculated that heat-sensitive ion channels or a change in conductance of ion channels may be behind the response to the transient heating of neurons. The importance of transient heating was also shown by Rajguru et al. (2011), who investigated INS of adult oyster toadfish *crista ampullaris*. Exposure to 1962 nm IR radiation resulted in an increase in the firing rate, while the application of indirect heat did not.

Following the proposal of Wells et al. (2007a) that heat-sensitive ion channels may be a mechanism behind INS, the heat-sensitive vanilloid subfamily of TRP channels (TRPV) have been the subject of some research in the literature (Suh et al., 2009; Katz et al., 2010; Albert et al., 2012). The TRPV channels, along with the TRPM subfamily, have the potential to detect changes in temperature from 10 to 50°C (Voets et al., 2005; Montell, 2005). Different TRPV channels activate at different temperatures, > 25°C for TRPV4, > 31°C for TRPV3, > 43°C for TRPV1, > 52°C for TRPV2 (Voets et al., 2004). Additionally, this temperature dependence is affected by the membrane voltage (Talavera et al., 2008). Expression of TRPV channels varies in different tissue (Montell, 2005) and could potentially explain some variations between different neural targets. Suh et al. (2009) examined the potential role of the TRPV1 channel, using knockout mice that lack the TRPV1 gene. The knockout mice showed an increase in acoustic thresholds of approximately 20 dB
over control. When stimulated optically, a response could only be evoked in two out of five knockout mice. Those that did respond to optical stimulation displayed an increase in thresholds over control. The results suggest that heat-sensitive ion channels, such as TRPV1, play a role in the neural response but are not the only factor in the response observed. Additionally, Rhee et al. (2008) exposed dissociated neurons from the vagus nerve of rats to laser stimulation and measured the change in intracellular Ca\textsuperscript{2+} concentrations. When a TRPV1 channel blocker, capsazepine was added, the laser exposure no longer generated a Ca\textsuperscript{2+} transient from an influx of Ca\textsuperscript{2+}.

Further work on TRPV channels was reported by Albert et al. (2012). The response of retinal and vestibular ganglion cells to $\lambda = 1875$ nm light was examined using whole cell patch clamp recording. The influence of TRPV channels was determined by adding various channel blockers, to remove their contribution to the response. When the TRPV4 channel was blocked, a response to the laser could no longer be produced, suggesting that TRPV4 channels play a role in neural activation from INS.

The response of intracellular calcium during INS was investigated by (Dittami et al., 2011), here a $\lambda = 1862$ nm diode laser was used to stimulate rat ventricular cardiomyocytes and the resultant intracellular calcium wave was imaged using a confocal microscope. The authors found that TRPV channel blockers 2-APB and Ruthenium Red blocked the intracellular release in response to INS. However, CGP-37157, an inhibitor of mitochondrial Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (mNCX) also blocked the response observed due to exposure to IR. The authors concluded that the mitochondria are the primary facilitator of the IR-evoked Ca\textsuperscript{2+} transients.

Recently Shapiro et al. (2012) have shown that the membrane capacitance varies reversibly during rapid heating, such as occurs during exposure to INS. The authors propose that INS is mediated by this shift in capacitance as it causes a change in membrane voltage that can initiate an action potential. Additionally the authors used heavy water to demonstrate the role of water as the primary chromophore absorbing laser irradiation during INS. Heavy water has approximately 20% the absorption coefficient of normal water at the wavelength investigated (1889 nm). When standard water was replaced by heavy water, the observed response reduced by 80%, demonstrating that light is primarily absorbed by water during INS. Oocytes, HEK cells and artificial lipid bilayers were exposed to 1869 nm (HEK cells) or 1889 nm laser light (oocyte and bilayers) and the response was recorded with a patch clamp. Recordings of the temperature shift using micropipette resistance measurements displayed a similar shape to the change in membrane voltage, as
1.4. INS Applications

Figure 1.10: Example of temperature measurement (left) and change in cell membrane potential (right) during and after exposure to INS. Figure from (Shapiro et al., 2012) is copyright Nature Publishing Group and is used with permission.

shown in the example in Figure 1.10. The observed change was consistent with a model of double layer capacitance and shows how the membrane voltage changes proportionally to the temperature.

Peterson and Tyler (2012) applied the changes in membrane capacitance observed by Shapiro et al. (2012) for the simulation of neurons to assess whether this mechanism would be adequate to explain the response observed in vivo and if myelination would change the response. They found that the capacitive mechanism was unable to activate neurons on its own and is unlikely to be the only mechanism behind INS. Additionally, they found that smaller fibres would have a lower activation threshold.

Roth et al. (2013) found that cells exposed to nanosecond pulsed electric fields created nanopores in the cellular membrane, leading to an influx of Ca\(^{2+}\). The authors speculate that the response seen due to exposure to INS may also be due to the formation of temporary, subtle disturbances in the cell membrane, based on the similarity in observed electrophysiological response.

Despite considerable progress towards understanding the mechanism behind INS, the details of the process have not yet been fully explained and the topic is still subject to further research. However, the current evidence points to changes in membrane capacitance underlying the primary response observed, with TRPV channels playing an additional modulatory role.

1.4.9 Modelling of INS

Recently, both analytical and numerical models have been developed to assist in understanding the flow of heat during INS, in the cochlea and in vitro models. As INS depends on a thermal gradient in time (Wells et al., 2007a; Shapiro et al., 2012), understanding the heat distribution in tissue may help to optimise the process and assist in emitter design. Modelling may also be to provide detailed thermal information for in vitro studies aimed at uncovering the mechanisms behind INS.
Chapter 1. Introduction

A numerical multiphysics model of heating during INS *in vitro* was developed by Liljemalm et al. (2013). Using an optical fibre with 200 µm core diameter and NA = 0.39, wavelength of 1550 nm, laser power of 300 mW and pulse length of 20 ms, the model predicted a temperature increase of 13.7 °C at the centre of the beam, 300 µm from the fibre emitter. When using multiple pulses at 10 Hz, the peak temperature increased by a further 1.7°C, stabilising after four pulses. Results from the model compared well with local temperature measurements using changes in current in a micropipette.

Norton et al. (2013) developed a Green’s function analytical model of thermal changes during INS. Rather than investigate temperature distributions, the model was applied to understanding what thermal changes are required to activate neurons, specifically the cochlea. The authors hypothesised that two thermal criteria are required for neural activation: a minimum temperature increase $T_c$; and a minimum temperature rate of change $\dot{T}_c$. By optimising these criteria, a pulse length can be found that satisfies both, thus reducing wasted energy. Experimental *in vivo* data of INS in the cat cochlea was provided by Richter et al. at Northwestern University. Using this data in combination with a CAP growth function gave values of $T_c = 0.8$ m°C and $\dot{T}_c = 15.1$ °C.s$^{-1}$ for the thermal criteria and a optimal pulse length of 53 µs. Further use of this approach to determine optimal pulse lengths may assist in developing more efficient INS implants.

1.5 Thesis Outline

The research presented in this thesis is organised into two parts and is summarised below:

Part I Modelling of INS The first part of the thesis introduces a thermal model of INS and investigates its application to better understand heat distribution and flow during INS.

Chapter 2 Modelling the spatial behaviour of INS This chapter investigates the spatial distribution of heating during INS using a Monte Carlo model to simulate light transmission and absorption in tissue. A wide range of optical fibre core diameters have been used to deliver wavelengths with varying water absorption properties. The model allows for the spatial heating patterns from these variations to be better understood.

Chapter 3 Modelling the temporal behaviour of INS This chapter introduces heat conduction into the model by solving the heat equation
with a finite element model. Consideration of heat conduction allows the
temporal behaviour of heating to be investigated. The effects of different
pulse lengths, spatial localisation of heating and non-absorbing media are
studied and the potential of the model to provide further insights into the
mechanisms of INS are discussed.

Chapter 4 Modelling of INS with multiple pulses The model is extended
further to allow for pulse repetitions, multiple emitters and modulation
schemes, as would be found in a bionic device using INS. This extension
allows for the impact of varying core diameter, pulse rate, emitter spacing
and modulation scheme to be evaluated.

Part II Infrared Stimulation in the Cochlea The second part of the thesis
details experimental results of infrared stimulation in guinea pig cochleae.

Chapter 5 Experimental Materials and Methods This chapter presents
the lasers that have been developed and used for infrared stimulation ex-
periments and describes the experimental techniques used in the following
Chapter. The different radiant exposures, wavelengths and pulse shapes
of the lasers are compared.

Chapter 6 Exploring Infrared Stimulation of the Cochlea Here, results
of infrared stimulation in guinea pig cochleae are presented, based on the
use of a number of different laser sources. Effects of deafening the cochlea
are described and discussed in context with the published literature.

Chapter 7 Conclusion The final chapter of this thesis summarises the results
and findings of the thesis, suggests avenues for further work and discusses the
overall outlook.
Chapter 1. Introduction
Part I

Modelling of INS
Modelling the spatial behaviour of INS

A wide range of optical fibre core diameters (20 – 1000 µm) and numerical apertures (0.14 – 0.39) have been used to deliver many different wavelengths (1450 – 2120 nm) of infrared light for INS. These various parameters deliver a large range of spatial intensity patterns to the target tissue, especially when the varying distances between the light emitting fibre and the target neurons are taken into consideration.

To better understand this variation, this chapter introduces a Monte Carlo model to simulate the photon transport and absorption in tissue during INS. Section 2.1 introduces Monte Carlo modelling, photon transport in tissue and the MCML method used here. Section 2.2 discusses the parameters selection for used for simulating photon transport during INS. The overall results of the model are shown in Section 2.3 and a discussion of the different parameters used is in Section 2.4.

Portions of this chapter have previously been published in the following publications (Thompson et al., 2012, 2013c,a).

2.1 Monte Carlo

Monte Carlo methods refer to a set of techniques first proposed by Metropolis and Ulam (1949) and which are now commonly used to simulate physical processes using a stochastic model. In related work, Wilson and Adam (1983) first introduced
Monte Carlo methods to study the propagation of light in tissues due to interest in photodynamic therapy (PDT). They presented a model using a homogeneous medium, with scattering and absorption coefficients of $\mu_s$ and $\mu_a$ respectively. The photons trace a randomly generated path with a mean free path length equal to $1/\mu_t$, where $\mu_t = \mu_s + \mu_a$ is the sum of the scattering and absorption coefficients. This model was extended by Prahl et al. (1989) to include anisotropy in scattering and internal light reflection upon reaching the boundary of the medium. While providing for interfaces with external media, the algorithm provided by Prahl et al. (1989) is limited in that it only allows for one medium to be modelled and doesn’t provide for multiple layers or more complex 3-D structures. To account for this Wang et al. (1995) extended the Monte Carlo model to allow for modelling of light transport in multi-layered tissues (MCML). Although many more sophisticated Monte Carlo models exist, which allow for discrete regions or voxelised tissues (such as Meglinski and Matcher (2002)), the MCML layered technique is adequate to investigate light behaviour during INS.

While use of this methodology has been extended and applied to various light/tissue interactions, it has yet to be used to study optical stimulation of neural tissue. Presented here, is a Monte Carlo model of light interaction during INS of various target nerves. For this work the MCML Monte Carlo model (Wang et al., 1995) was used, modified to make use of the multi-threading capability of OpenMP to allow multiple CPU cores to perform calculations on photon behaviour.

### 2.1.1 MCML Monte Carlo Model

This section provides an overview the MCML model as implemented for this work; for further details readers are referred to (Wang et al., 1995) for a full discussion on implementation.

Photons are launched from a multimode fibre, from a position $(x, y, z)$ within the fibre diameter, and with directional cosines $(xv, yv, zv)$ based upon the numerical aperture (NA) of the fibre and with an initial weight ($w$) of 1. The directional cosines always keep a unit value of 1 (i.e. $xv^2 + yv^2 + zv^2 = 1$). The $z$ plane is parallel to the optical fibre while the $x$ and $y$ are perpendicular to the fibre. The axes for each geometry are shown in Figures 2.4, 2.5 and 2.6. The distribution of the position and direction vectors is described in Section 2.2.1 and depends on the fibre used. Once launched, the photons iteratively move through the media until they are completely absorbed or exit past the boundaries of the simulation.

While iterating, photons are given a step size $s$ (denoted stepSize in the C++ code), where $s = -\ln(\xi)/\mu_t$, where $\xi$ is a random variable with uniform distribution
Figure 2.1: Flow diagram of Monte Carlo Simulation.
Chapter 2. Modelling the spatial behaviour of INS

over the interval of (0,1) and $\mu_t$ is the total interaction coefficient equal to the sum of absorption and scattering coefficients for that material ($\mu_t = \mu_a + \mu_s$). If multiple media are present, the step size could be greater than is left in the current media. If this happens, refraction and reflection at the interface will not be taken into account, nor will the different properties in the new media. To avoid this, an intermediate value $\bar{s} = -\ln(\xi)$ is used. If the distance from the photon to the next medium ($d_b$) is greater than than the step size ($d_b > \bar{s}/\mu_t$), the photon will remain in the current medium and moves to the new position based on the step size ($s = \bar{s}/\mu_t$), current position and direction:

$$
x \leftarrow x + xv \times s \\
y \leftarrow y + yv \times s \\
z \leftarrow z + zv \times s.
$$

(2.1) (2.2) (2.3)

The photon then reaches an interaction site and is then scattered and absorbed. If ($d_b < \bar{s}/\mu_t$), the photon is moved to the boundary of the medium and refraction and reflection is calculated. At the boundary, $\bar{s}$ is adjusted to take into account the distance travel in the previous medium $\bar{s} = \bar{s} - d_b\mu_t$ and is kept for the next movement iteration.

Refraction is calculated by using Snell’s law, based on the relationship between the angle of incidence and the refractive indices of the media that the photon is incident from ($n_i$) and transmitted to ($n_t$):

$$
n_i \sin \alpha_i = n_t \sin \alpha_t.
$$

(2.4)

Reflection is given from both total internal reflection, if $n_i > n_t$, and Fresnel reflection. If $\alpha_i$ is larger than the critical angle, the photon is reflected, due to total internal reflection. If the photon is refracted, the change of intensity due to Fresnel reflection is considered, with the average reflectance given by:

$$
R(\alpha_i) = \frac{1}{2} \left[ \frac{\sin^2(\alpha_i - \alpha_t)}{\sin^2(\alpha_i + \alpha_t)} + \frac{\tan^2(\alpha_i - \alpha_t)}{\tan^2(\alpha_i + \alpha_t)} \right].
$$

(2.5)

The chance of the photon being reflected is then calculated by comparing to it a random number ($\xi$), if $\xi \leq R(\alpha_i)$ the photon is internally reflected.

Once the photon reaches an interaction site, it is scattered and a fraction of its weight absorbed. Absorption is calculated by taking a fraction of the weight from the ratio of the absorption and scattering coefficients. The fraction of the photon
weight absorbed is given by:

\[ \Delta w = \left( \frac{\mu_a}{\mu_t} \right) w. \]  

(2.6)

and is stored in the 3D array to record the energy absorbed at the position of the photon, while the photon weight is updated to be:

\[ w \leftarrow w - \Delta w. \]  

(2.7)

If OpenMP is used to provide multithreading, each thread has its own independent 3D array, which are summed together at the end of the stimulation. This is to prevent multiple threads writing to the same memory structure, which will cause memory corruption.

Once the photon’s weight has been decreased at the absorption step, it is evaluated to determine whether it will continue or be terminated. To perform this the roulette technique is used, where a photon is killed if its weight drops below a minimum. If the weight has dropped below the minimum (here 0.001), it is given a one in \( m \) (here 10) chance of continuing with its weight multiplied by \( m \) (i.e. \( w_m \)) or terminated. This terminates photons when their weight is too low, without sacrificing energy conservation.

If the photon survives the roulette, the scattering of the photon is calculated. A deflection angle \( \theta \) and azimuthal angle \( \phi \) are generated. The deflection angle is described by the Henyey-Greenstein phase function which depends upon the anisotropy \( (g) \) of the media, where a value of 0 indicates isotropic scattering and a value near 1 indicates forward-directed scattering. The cosine of the deflection angle is given by

\[ \cos \theta = \frac{1}{2g} \left\{ 1 + g^2 - \left[ \frac{1 - g^2}{1 - g + 2g} \right]^2 \right\} \]  

(2.8)

unless \( g = 0 \), in which case, isotropic scattering means \( \cos \theta = 2\xi - 1 \).

The azimuthal angle is uniformly sampled over the interval \((0, 2\pi)\) and is given by:

\[ \phi = 2\pi \xi. \]  

(2.9)

Therefore the photon is deflected by an angle \((\theta, \phi)\) from its current direction.
(xv, yv, zv). The new direction (xv', yv', zv') is given by:

\begin{align*}
xv' &= \frac{\sin \theta}{\sqrt{1 - zv^2}} (xvzv \cos \phi - yv \sin \theta) + xv \cos \theta \\
yv' &= \frac{\sin \theta}{\sqrt{1 - zv^2}} (xyzv \cos \phi - xv \sin \theta) + yv \cos \theta \\
zv' &= -\sin \theta \cos \phi \sqrt{1 - zv^2} + zv \cos \theta.
\end{align*}

(2.10) (2.11) (2.12)

Once the photon is scattered, it returns to the top of the loop to be moved (Figure 2.1). This continues until the photon’s weight reduces and it is terminated, or it moves outside the pre-defined bounds.

The 3D array used for most of the work presented spanned a volume of 1×1×1 mm with a resolution of 2.5 µm to make a total size of 400×400×400 voxels. However, when the data is saved to disk the resolution is reduced to 5 µm to reduce the file size by an approximate factor of 8. To allow some data at maximum resolution to be analysed, a 2D slice in the middle of the y axis (x − z plane) is saved with a resolution of 2.5 µm. Although a 2D (r, z) cylindrical geometry would allow faster computation, the 3D geometry was required for non-symmetrical geometries such as the in vitro cell culture geometry (Section 2.2.5).

### 2.2 Parameter Selection for INS Modelling

As the wavelengths of light typically used for INS (1.45 µm < λ < 2.12 µm) have not been frequently studied in laser-tissue interactions, there is an absence in the literature of authoritative values of coefficients for scattering (\(\mu_s\)), absorption (\(\mu_a\)) and anisotropy (\(g\)) for tissue at these wavelengths. This section discusses the selection of coefficients used for various tissue types and how light was modelled to behave when exiting an optical fibre.

A summary of the media properties used is provided in Table 2.1.

<table>
<thead>
<tr>
<th>(\mu_a) (mm(^{-1}))</th>
<th>Perilymph</th>
<th>Bone</th>
<th>Nerve</th>
<th>Glass</th>
<th>Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu_a) (mm(^{-1}))</td>
<td>0.96</td>
<td>0.96</td>
<td>0.96</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>Refractive index (n)</td>
<td>1.33</td>
<td>1.33</td>
<td>1.33</td>
<td>1.45</td>
<td>1.0</td>
</tr>
<tr>
<td>Anisotropy (g)</td>
<td>-</td>
<td>0.85</td>
<td>0.85</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heat capacity (C_p) (kJ.kg(^{-1}.K(^{-1}))</td>
<td>4.18</td>
<td>4.18</td>
<td>4.18</td>
<td>0.75</td>
<td>1.006</td>
</tr>
</tbody>
</table>

Table 2.1: Parameters used for simulations when \(\lambda = 1850\) nm.
2.2.1 Optical Fibre Properties

Light output from the fibre is modelled using photons initialised with an even top hat distribution across the fibre end face. This is a valid approximation for a multi-mode fibre, as the normalised frequency for the smallest core fibre studied here is much greater than 1 (Snyder and Love, 1983), meaning all fibres of interest are multimode.

The divergence of light from the core, given by the numerical aperture (NA), is simulated by an even spread of angles between 0 and the critical angle. The spread of light out of a multimode fibre can be described by \( P = 2n_{co} \cos \theta_z / NA^2 \), where \( P \) is the intensity at a given angle \( \theta_z \), in the range \( 0 < \theta_z < \theta_{\text{max}} \) (Snyder and Love, 1983). The intensity distribution is very close to the light rays being evenly distributed equally in all directions up to the maximum angle. While the NA quoted here is for the fibre in air, this is modified by the refractive index of the media when the fibre is placed in a non-air environment, such as the perilymph in the inner ear. This change in refractive index reduces the effective NA, and has been considered when simulating relevant geometries.

A range of different fibre types have been used to deliver light to target tissue in INS. The most commonly used fibre has a 200 \( \mu \text{m} \) core diameter and NA of 0.22. The most common range of diameters extends from 100 \( \mu \text{m} \) (Izzo et al., 2006) up to 600 \( \mu \text{m} \) (Wells et al., 2005b), however there are examples of single mode fibres and core diameters of up to 1000 \( \mu \text{m} \) being employed during INS experimentation in the literature (McCaughey et al., 2010). The range of NAs is typically between 0.22 - 0.39, but has extended down to 0.14 for single mode fibres.

Here the typical range of 100 – 600 \( \mu \text{m} \) core diameters will be considered, along with an NA range of 0.14 – 0.44. Figure 2.2 illustrates the variation in fibre core diameter and numerical aperture (NA) and the effect it may have on the resultant light profile.

2.2.2 Absorption Coefficient

In the wavelength range where water strongly absorbs light (1400 - 2500 nm), it dominates the absorption characteristics of biological materials, as the water concentration is typically 70% (Niemz, 2007) and there are no other dominant chromophores present in tissue that absorb these wavelengths. Therefore, values of the absorption coefficient (\( \mu_a \)) in biological media are generally assumed to be the same as those for water absorption (Curcio and Petty, 1951; Hale and Querry, 1973). In addition the differential between tissue and water decreases at longer wavelengths (Troy and Thennadil, 2001) and the absorption difference between water and blood
Figure 2.2: Variation in fibre core diameter and NA.
is minimal (Roggan et al., 1999). Although absorption could be lower in tissue than pure water, as the water content of tissue varies and is typically 70% (Niemz, 2007), the absorption of light in water is taken here as a first approximation of the absorption of light in tissue. The effect of variations in this value are considered in Section 2.4.3.

The change in water absorption and the resultant penetration depth over the 1400 – 2500 nm wavelength range is shown in Figure 2.3. This range includes wavelengths of 1850 nm, 1870 nm and 2120 nm that are commonly used for INS. These common wavelengths are marked to highlight their absorption, along with the absorption band in the range 1 – 2 mm\(^{-1}\). For fused quartz and glass a nominal absorption coefficient of \(\mu_a = 0.01\) was used.

![Figure 2.3: Coefficient of absorption (\(\mu_a\)) and penetration depth for water in the 1400 – 2500 nm wavelength range, covering the wavelengths most frequently used for INS. The absorption range of 1 – 2 mm\(^{-1}\) is highlighted to show the range of absorption values commonly used, together with the commonly used wavelengths of 1850 nm, 1870 nm and 2120 nm. Data derived from (Curcio and Petty, 1951; Hale and Querry, 1973).](image)

### 2.2.3 Scattering Coefficient

Due to the lack of good quality data on scattering in tissue at the wavelengths of light typically used for INS (1450 nm < \(\lambda\) < 2120 nm), values for the model presented here have been derived from the available sources and by extrapolating the trends in scattering at wavelengths that have been more frequently studied (Vo-Dinh, 2003).

Typically scattering decreases at longer wavelengths (Niemz, 2007), such as mid-infrared wavelengths, compared to near-infrared wavelengths where there have been more measurements of tissue scattering (Vo-Dinh, 2003). Scattering in tissue is not well described by either the Rayleigh process (Niemz, 2007), which has a
wavelength dependence of $\mu_s \sim \lambda^{-4}$, or Mie scattering, with a wavelength dependence of $\mu_s \sim \lambda^{-0.4}$. This makes it hard to extrapolate scattering values to different wavelengths.

Troy and Thennadil (2001) presented scattering coefficients for skin at wavelengths of 1000 – 2200 nm. Values for scattering were provided using the isotropic scattering constant ($\mu'_s$), which can be converted back to the scattering constant ($\mu_s$) through the anisotropy factor $g$: $\mu_s = \frac{\mu'_s}{(1-g)}$. At 1.8 $\mu$m, Troy and Thennadil (2001) give a range of $\mu'_s = 0.45 - 0.98$ mm$^{-1}$, which when using an anisotropy factor of $g = 0.85$, gives a scattering coefficient of $\mu_s = 3 - 6.5$ mm$^{-1}$.

Although there is a wide variation in values for the coefficient of scattering, even in well studied tissues and wavelengths, scattering in neural tissue appears to be approximately 2-3 times lower than that of skin at around 800 nm (Vo-Dinh, 2003). Therefore values used for neural tissue in the model are set to $\mu_s = 1$ mm$^{-1}$. However, variations in this scattering value and the sensitivity of the model to variation in scattering are considered in Section 2.3.1.

In the case of the cochlea, the contribution to scattering of light by the osseous spiral lamina also needs to be considered, as any light from an emitter in the scala tympani needs to pass through the osseous spiral lamina to reach the spiral ganglion neurons. Scattering for bone at wavelengths of 650 – 950 nm is given by Firbank et al. (1993), in the range of $\mu_s = 25 - 35$ mm$^{-1}$. While Bevilacqua et al. (1999) gives values of $\mu_s = 6 - 9$ mm$^{-1}$ for skull in the same wavelength range. Given the assumption made previously (2 – 3 times reduction) about the change in scattering behaviour at longer wavelengths, bone was given a scattering value of $\mu_s = 2$ mm$^{-1}$.

### 2.2.4 Heat Capacity

Tissue has a range of specific heat capacity, primarily depending on the water content of the tissue. An approximation for most tissues is given by:

$$C = \left(1.55 + 2.8 \frac{\rho_w}{\rho} \right) \text{kJ.kg}^{-1}.\text{K}^{-1},$$

where $\rho$ is the overall tissue density and $\rho_w$ is density of the water content in the tissue (Niemz, 2007). Typical water content for tissue is in the range of 70 - 85% (Giering et al., 1995). For simplicity the specific heat capacity of water (4.18 kJ.kg$^{-1}$.K$^{-1}$) is used in the present model. If the nerve was only 60% water (e.g. $\frac{\rho_w}{\rho} = 60\%$) rather than 100% as assumed, the temperature increase could be 1.29 times higher, due to the lower specific heat capacity. Variations in the specific heat capacity due to a reduction in the water content are considered in this chapter. However, any reduction
2.2. Parameter Selection for INS Modelling

in the specific heat capacity is likely to be matched by a corresponding reduction in
the absorption coefficient of water, resulting in roughly comparable temperatures.

The energy absorbed per unit volume can be converted to change in temperature
from baseline, by using the specific heat capacity and density of material.

\[ \Delta T = \frac{E}{C_p V \rho}, \]  

(2.13)

where \( E \) is the energy delivered to a voxel, \( C_p \) is the specific heat capacity of the
material, \( V \) is volume of a voxel and \( \rho \) is the density of the material. Unless stated,
the specific heat capacity of water \( (C_o = 4.18 \text{ kJ.kg}^{-1}.\text{K}^{-1}) \) is used.

While this does not take into account changes due to heat conduction, it provides
a reasonable estimate of the change in temperature, as the thermal penetration depth
\( (z_{\text{therm}} = \sqrt{4 \alpha t}) \) during a 60 \( \mu \text{s} \) pulse is only 5.8 \( \mu \text{m} \) (where \( \alpha = 1.4 \times 10^{-7} \text{ m}^2.\text{s}^{-1} \) is
the thermal conductivity of water and is approximately the same for liquid water
and most tissues (Niemz, 2007)).

2.2.5 Geometry

The geometry of different target neurons may change the amount of light absorbed at
the target, both through absorption before the target and scattering causing a spread
of light. This change in absorption may result in a change in the thermal behaviour
and result in differences in the effectiveness of INS. Three different geometries are
considered here, namely a simple representation of the cochlea nerves, the sciatic
nerve and an in vitro cell culture experiment. These geometries were selected as
INS of the cochlea is the most commonly studied single target; differences between
different targeted peripheral nerves is minimal; and in vitro studies are important in
providing an understanding of the mechanisms behind INS, so further information
about the spatial temperature resulting from stimulation could be beneficial to
unravelling the biophysical mechanisms. Unless stated, the cochlea model was used,
as stimulation of the cochlea is the focus of this thesis.

Cochlea Model

As one of the primary targets of INS are the spiral ganglion neurons in the cochlea, a
geometry that approximates the guinea pig cochlea has been developed. This model
is designed to simulate INS of nerves in the modiolus of a guinea pig cochlea, where
an optical fibre is inserted inside the cochlea through a cochleostomy to direct light
towards the spiral ganglion cells. The guinea pig was selected because much of the
experimental work in this thesis was performed on guinea pigs and much of the
literature has used guinea pigs or gerbils, which are of a similar size. The model is representative of the approach used by Richter et al. (2011b) and Moreno et al. (2011). Although other approaches to the spiral ganglion nerves in the modiolus have been made, typically through the round window (Izzo et al., 2007a), this approach is broadly representative of the behaviour of light when targeting these nerves.

The 3D geometry of the model is made of slab layers of perilymph, bone and nerves, as shown in Figure 2.4. Clearly this geometry represents a considerable simplification of the complex 3D structure (Shepherd et al., 2005), including the fact that bundles of the central processes of the spiral ganglion neurons are known to project through pores in the osseous spiral lamina on the modiolar side of Rosenthal’s Canal (Shepherd and Colreavy, 2004). However, this simplification takes into account the known dimension of the guinea pig cochlea (Shepherd et al., 2005) and is useful in understanding the general principles behind light delivery to this region.

![Figure 2.4: Geometry used to represent the guinea pig cochlea for Monte Carlo simulations (not to scale).](image)

**Sciatic Nerve**

INS studies on peripheral nerves are typically performed with the nerve exposed to the environment (Wells et al., 2007a; Duke et al., 2012a). This differs to the cochlear stimulation as there is an air-tissue interface, as the nerve is typically exposed to the atmosphere, rather than water-tissue interface in the case of cochlear stimulation. Potential consequences of this difference are changes in beam diameter at the target neurons due to greater expansion of the beam, and evaporation of water, which would transport heat away from the tissue. Figure 2.5 shows the geometry used for stimulation of the sciatic nerve, here a flat nerve layer is separated from an optical fibre by distance $d_{\text{fibre}}$, using a similar slab geometry to that used for the cochlea model (Fig 2.4).
2.2. Parameter Selection for INS Modelling

Figure 2.5: Geometry used for Monte Carlo simulations of sciatic nerve stimulation (not to scale).

Cell Culture

In vitro work has been important to help uncover the biophysical mechanisms behind INS (see Chapter 1 for a full discussion). To advance this understanding, it is helpful to simulate the distribution of light directed from optical fibres to cells under investigation. As many of the target cells are approximately 10 μm in diameter, small variations in the position of an optical fibre may have a large influence on the resulting energy delivered to the target neurons. A variety of different geometries have been used for in vitro investigations of INS, such as those used by Brown et al. (2013), Shapiro et al. (2012) and Albert et al. (2012).

Figure 2.6: Cell culture geometry used for Monte Carlo simulations (not to scale).

The arrangement used by Brown et al. (2013) was examined, as shown in Figure 2.6. For this work, spiral ganglion neurons were attached to a 300 μm thick glass coverslip, which was immersed in a fluid bath with a polycarbonate base. As access for both the patch clamp pipette and optical fibre were required, they were inserted at an angle of approximately 35° for the pipette and 36° for the optical fibre.
2.3 Monte Carlo Modelling of INS

Figure 2.7 shows the typical characteristics of light absorption in the model cochlear geometry, using a 2D slice of the Monte Carlo simulation. For this image, the total pulse energy launched was 25 $\mu$J, equivalent to a radiant exposure of $\sim 80$ mJ.cm$^{-2}$ at the fibre tip, roughly twice the threshold required to elicit a 50 $\mu$V CAP in control gerbils for a pulse 60 $\mu$s in duration (Richter et al., 2008). Other parameters used were: $\lambda = 1850$ nm, NA = 0.22, core diameter = 200 $\mu$m, $n_{\text{photons}} = 1 \times 10^{11}$. White vertical lines show the bone layer between 440 and 450 $\mu$m and the nerves between 450 and 550 $\mu$m. Beyond 550 $\mu$m the medium has the coefficients of perilymph. From Figure 2.7 it can be seen that for the geometry and parameters used here, scattering in the bone and nerve has a negligible effect on photon propagation compared to absorption. As the majority of wavelengths used for INS have an absorption coefficient similar to or exceeding the scattering coefficient, this behaviour is expected to be consistent across all wavelengths used.

Figure 2.7: Example of a Monte Carlo simulation where an optical fibre is positioned 500 $\mu$m from the centre of the nerve layer. The temperature increase is greater near the fibre than further away and that light spreads out from the fibre. White vertical lines show the bone layer between 440 and 450 $\mu$m and the nerves between 450 and 550 $\mu$m ($\lambda = 1850$ nm, NA = 0.22, $\varphi_{\text{core}} = 200$ $\mu$m, $n_{\text{photons}} = 1 \times 10^{11}$, pulse energy = 25 $\mu$J).

An example of modelling the change in temperature due to optical stimulation is shown in Figure 2.8. It presents the same data as Figure 2.7 converted to the change in temperature using the specific heat capacity of water. In the nerve layer, the temperature increase is around 0.1 °C, with a 0.03 °C difference in temperature across the nerve layer.
Comparison with experimental results

An estimate of the accuracy of the Monte Carlo simulations and the subsequent conversion to temperature changes can be made by comparing results measured by Wells et al. (2007a). Simulating a 600 µm core fibre with a wavelength of 2120 nm and a 1.13 mJ pulse (corresponding to 0.4 J.cm\(^{-2}\) exposure), was found to create an average temperature rise of \(\sim 2.2^\circ\)C. This temperature rise is similar in magnitude to the 3.66 °C average rise measured with a thermal camera at the end of the pulse (Wells et al., 2007a).

Another comparison can be drawn from Shapiro et al. (2012) who used a 1889 nm laser coupled into a 400 µm core diameter fibre to stimulate oocytes in water on a glass coverslip, with the fibre located below the coverslip aimed up towards the oocyte. A number of pulse lengths and energies were used: a 1 ms, 2.8 mJ pulse gave a temperature increase of 15.2 °C and a 2 ms, 5.6 mJ pulse gave a 27.1 °C increase, when measured with a pipet resistance technique at a distance of approximately 100 µm from the coverslip. A simulation with the same parameters for a 2.8 mJ pulse, gives a temperature increase of 24.1 °C next to the slide and 13.9 °C at a distance of 100 µm. When the energy is increased to 5.6 mJ, the temperature range is 48.2 – 27.8 °C at the two positions given. This result is again similar in magnitude to the experimental values, the discrepancy may to be due to thermal conduction, which is considered in Chapter 3.

Overall, these results show a good correspondence between the model and experimental results given the difficulties in determining the exact position from which a measurement was made.
Table 2.2: Comparison between model and published experimental measurements. The predicted temperature range for the Shapiro et al. (2012) results accounts for an increase in distance of up to 100 µm from the coverslip.

2.3.1 Sensitivity to parameter variation

Scattering

As discussed in Section 2.2.3, the literature lacks reliable values of scattering for the wavelength range typically used for INS. A selection of $\mu_s = 1$ mm$^{-1}$ for tissue was made as an initial estimate of the scattering that is expected in tissue. With an anisotropy of $g = 0.85$, this gives a reduced scattering coefficient of $\mu'_s = 0.15$ mm$^{-1}$. The reduced scattering coefficient is given by $\mu'_s = \mu_s(1 - g)$. However, it is worth considering the effect of varying the scattering value to see what influence it has on the resulting temperature achieved.

![Figure 2.9](image-url)

Figure 2.9: Shows the change in temperature in the nerve layer for an optical fibre ($\lambda = 1850$ nm, $\phi_{\text{core}} = 200$ µm, NA = 0.22, $E_{\text{pulse}} = 25$ µJ) located 500 µm away. The x-axis displays the reduced scattering coefficient ($\mu'_s = \mu_s(1 - g)$) in the nerve layer; the scattering in the bone is always set to 2 times that of the nerve.

Figure 2.9 shows the temperature increase in the nerve region for a 200 µm core diameter fibre located 500 µm from the nerve. The reduced scattering values shown on the x-axis are for the nerve, while scattering in the bone is kept at 2 times that of the nerve. Increases in scattering initially cause a small increase in the temperature achieved in the nerve. A reduced scattering value of 1.5 mm$^{-1}$ gives
approximately the same peak temperature as $\mu_s' = 0.15 \text{ mm}^{-1}$. Beyond this, the relative temperature decreases due to light being scattered away from the target tissue.

![Figure 2.10: Temperature change achieved along the axis of the fibre for different scattering values of bone and nerve. The values displayed are for nerve, while the bone is kept at 2 times that of nerve. Irregularities in the data are due to statistical variation, inherent to the Monte Carlo process. Lines at 440 $\mu$m and 450 $\mu$m show the bone layer and lines at 450 $\mu$m and 550 $\mu$m show the nerve layer. Beyond the nerve layer, the medium is water (i.e. $\mu_s = 0$).](image)

The change due to different scattering coefficients can be more clearly seen by plotting the temperature along the optical fibre axis for different levels of scattering as shown in Figure 2.10. This shows the change across the nerve layer as the scattering increases. Beyond the nerve layer ($z = 550 \mu$m), the medium is water (i.e. $\mu_s = 0$). The reduction in temperature for ($\mu_s = 1 \text{ mm}^{-1}$) at $z \sim 600 \mu$m is due to expansion of the beam from the optical fibre. For the scattering values most likely to be present in the tissue of interest ($1 < \mu_s < 10 \text{ mm}^{-1}$) there is only a small change in the temperature achieved, with a slightly higher values at the start of the tissue layers and slightly lower at the end.

As the scattering continues to increase, the energy in the tissue layers diffuses further and less is absorbed in the target neurons. When $\mu_s = 100 \text{ mm}^{-1}$ the temperature increase at the end of the nerve layer ($z = 550 \mu$m) is reduced to 1/6 of the $\mu_s = 1 \text{ mm}^{-1}$ case. However, at the start of the tissue layer an increased temperature of 25% is observed due to back scattered light.

These results show that for the cochlear case, in the range of scattering values that are feasible to be present at these wavelengths, the assumptions made regarding the scattering are reasonable and provide a good first approximation.
2.4 Modelling of Spatial Behaviour

The greatest advantage of Monte Carlo techniques is that they can take into account the expansion of the beam and non-uniform shapes or geometries, such as where the fibre is on an angle. Rudimentary calculations of the radiant exposure typically do not allow these factors to be taken into account. However, it is still beneficial to see whether these calculations provide a reasonable estimate of the behaviour of light.

The temperature change from exposure to a short laser pulse, on the axis of the beam, can be considered through use of (1), a basic analytical solution for the temperature from a collimated beam and instantaneous pulse (Izzo et al., 2008a; Niemz, 2007):

\[
T(z,0) = \frac{\mu_a H(z)}{\rho C_p} \quad (2.14)
\]

where \(\rho\) is the density of the medium, \(C_p\) is the heat capacity and \(H(z)\) is the radiant exposure at distance \(z\). \(H(z)\) is given by the Beer-Lambert law:

\[
H(z) = H(0)e^{-\mu_aoz} \quad (2.15)
\]

where \(H(0)\) is the radiant exposure at the fibre tip. The applicability of this solution for more complex geometries can be considered by comparing it to the output from the Monte Carlo (MC) model, such as that shown in Figure 2.8.

![Figure 2.11](image_url)

Figure 2.11: Diagram showing how the radiant energy remains localised on the fibre axis until \(z = \frac{\varnothing_{\text{core}}}{2\tan(NA/n)}\). Light expanding at the maximum direction from the edges of the core is marked with a grey line. Figure shows a fibre with \(\varnothing_{\text{core}} = 200 \, \mu m\), NA(air) = 0.22, placed in a non-absorbing medium with a refractive index \(n = 1.33\).

Figure 2.11 shows the expansion of light from a fibre, in a medium with no absorption or scattering. Grey lines show the maximum angle of expansion in both
directions. It clearly shows how light expands away from the fibre core, unlike that considered in simple analytical models with a collimated beam. Near the fibre, there is a region of relatively uniform energy, centred on the fibre axis, where the radiant energy can be predicted by the Beer-Lambert law. In this region, the loss of light expanding in one direction is matched by light gained from the other direction. Beyond this region, the radiant energy is less than the absorption loss predicted by the Beer-Lambert law for a collimated beam. This transition point is given by \[ z_T = \frac{\Phi_{core}}{2 \tan(\sin(NA/n))} \]. When comparing between different fibre diameters, it is helpful to keep the transition point \( z_T \) constant. A constant distance to the transition point ensures that differences in temperature at the neurons are not due to varying radiant exposures, but are driven by changes in the localisation of light. It is also worth noting that this effect will be more pronounced in air, as the effective NA of a fibre in water is reduced as \( NA_{eff} = \frac{NA}{n} \).

This localisation transition can be seen more clearly by plotting the temperature along the fibre axis expected due to an instantaneous pulse. Figure 2.12 shows the temperatures predicted by Eq. (1) and for the Monte Carlo model using 100 µm and 200 µm core diameter fibres with NA of 0.11 and 0.22 and refractive index of \( n = 1.33 \).

![Figure 2.12: Comparison of the analytically predicted temperature rise along the optical fibre axis and Monte Carlo simulation results at \( \lambda = 1850 \) nm, \( N_{photons} = 10^{11} \), \( n = 1.33 \), using the guinea pig cochlea model, fibre core diameters of 100 µm and 200 µm and NA of 0.11 and 0.22. Radiant exposure \( (H(0)) \) is the same for all examples.](image)

The results in Figure 2.12 show that a fibre with a core diameter of 100 µm has similar localisation compared to a fibre with a core diameter of 200 µm and NA = 0.22 when the 100 µm core diameter fibre’s NA is reduced to 0.11. While it may not be practical to specify fibre design so precisely, similar effects to varying the
NA can be achieved by use of lenses to focus or disperse light. To allow convenient comparison between different fibre core diameters, the NA can be selected to keep $\tau_T$ constant.

These results show the region in which simple analytical models are still able to provide an accurate estimation of temperature, and where use of more advanced models are helpful in fully understanding the thermal distribution in tissue. It also shows how optimising fibre design and stimulation wavelengths can assist in designing an emitter which ensures stimulation is localised in the longitudinal direction as well as the transverse, while also not requiring very small distances between the emitter and target nerves as the case when using more strongly absorbing wavelengths (e.g. 1937 nm (Izzo et al., 2008a)). Careful use of modelling when designing optical emitters for INS allows localisation of light regardless of the wavelength and allows the risk of cross talk between different stimulation channels to be evaluated (see Section 4.2.2).

2.4.1 Fibre Diameter

In this section the effect of different fibre diameters is investigated using the guinea pig cochlea model presented before. Although the overall range of fibre diameters that have been used experimentally for cochlea stimulation is smaller than the range reported for other INS experiments, it is still worth considering the effect of different sized fibres with this geometry due to the small effect of scattering in tissue at these wavelengths. Fibres used for INS studies have typically been selected to balance ease of coupling of laser light into the core (larger sized core), or maximising the intensity of light at the emitting end (smaller cores). Standard silica multimode fibre (core diameter = 200 µm) is the most commonly used for INS (Richter et al., 2011a). However, other fibres such as hollow core fibres have also been used (Wells et al., 2005a) in cases where the absorption in silica is too high at the wavelength chosen for particular stimulation study.

An example of the heat distribution resulting from 1850 nm light delivered by 200 µm and 100 µm core diameter fibres, with NAs of both 0.22 and 0.11, is shown in Figure 2.13. An energy of $E = 25 \mu J$ was used in the $\varnothing_{\text{core}} = 200 \mu m$ fibres and $E = 6.25 \mu J$ for the $\varnothing_{\text{core}} = 100 \mu m$ to keep a constant radiant exposure of 80 mJ.cm$^{-2}$ at the fibre tip. The figure clearly shows that smaller core diameters keep the light more localised, in all dimensions. The $\tau_T$ distance also reduces with fibre diameter, if the NA is held constant.

The effect of fibre diameter on spot size at the nerve and the resulting temperature can be more clearly investigated by measuring the spot size predicted by the model
2.4. Modelling of Spatial Behaviour

Figure 2.13: Figure showing temperatures achieved with fibre diameters of \( \varnothing_{\text{core}} = 100 \, \mu m \) and \( \varnothing_{\text{core}} = 200 \, \mu m \) with NA = 0.11 and NA = 0.22. For \( \varnothing_{\text{core}} = 100 \, \mu m \), \( E_{\text{pulse}} = 6.25 \, \mu J \) and for \( \varnothing_{\text{core}} = 200 \, \mu m \), \( E_{\text{pulse}} = 25 \, \mu J \), giving the same radiant exposure in all cases.)
in the nerve layer and by taking the temperature at a point in the middle of the nerve layer. Figure 2.14 shows the relationship between the spot size, calculated with both full width half maximum (FWHM) and $\frac{1}{e^2}$ of the maximum temperature and the temperature increase in the middle of the nerve layer (righthand y axis). A wavelength of 1850 nm with a pulse energy of 25 $\mu$J was used for all diameters. The fibre was positioned 500 $\mu$m from the centre of the nerve.

Figure 2.14a shows the increase in spot size as the core diameter of the optical fibre increases ($\text{NA} = 0.22$). As the core diameter increases the $\frac{1}{e^2}$ spot size also increases, however the FWHM spot size only increases with core diameters greater than $\sim 200 \, \mu$m. This is due to the expansion of light from the core behaving as the dominant factor below $\varphi_{\text{fibre}} \sim 200 \, \mu$m as the $z_T$ point is before the target. For larger diameters the core diameter of the fibre has more influence on the resulting spot size.

The effect of changing core diameter while keeping the $z_T$ distance constant at 600 $\mu$m can also be considered. Holding $z_T$ constant serves to keep the profile of the beam constant in the targeted region of neurons and reduces the effect of expansion of the beam (which is a larger factor for smaller core diameter fibres). Figure 2.14b shows the the change in spot size as the core diameter is varied and the resulting peak temperature. Here, the spot size has a linear dependence upon the core diameter over the full range of diameters, as the core diameter now has the strongest influence
2.4. Modelling of Spatial Behaviour

2.4.2 Fibre Numerical Aperture

Figure 2.15 shows the relationship between fibre NA and the laser spot size, while using constant core diameters of $\phi_{\text{core}} = 200 \, \mu\text{m}$ (a) and $\phi_{\text{core}} = 100 \, \mu\text{m}$ (b). When the core diameter is held at $\phi_{\text{core}} = 200 \, \mu\text{m}$ (Fig 2.15a), it is seen that the $1/e^2$ spot size increases linearly with increasing NA, due to the larger divergence of the

on the spot size. The temperature shows much greater variation, as the smaller core diameters also have a reduced NA and therefore spread of light from the core, resulting in the energy being kept much more localised.

These results show that, as expected, smaller core diameters keep the light more localised. However, the resulting spatial distribution from smaller core diameters is more dependent upon the spread of light, due to the reduced $z_T$ distance. This makes the temperature distribution diverge from the prediction of a simple analytical case. Additionally, this means smaller core diameters require more precise positioning, due both to targeting the neurons in the x-y axes and changes in the z axis. Additionally, for smaller core diameter fibres with moderate or large divergences, a much higher intensity is required close to the fibre to achieve a given temperature increase at the target $500 \, \mu\text{m}$ away. This higher intensity may damage tissue between the fibre and the target and could place a limit on the maximum safe rate of stimulation that can be achieved. Additionally, factors such as laser coupling efficiency may also need to be taken into account when selecting a fibre delivery system.
beam, while the FWHM spot size only increases with an NA greater than 0.22. The increase beyond an NA of 0.22 is due to the $z_T$ transition distance reducing to less than 500 $\mu$m, which means that the light is less confined and spreads over a wider region. This trend is confirmed by the reduction in peak temperature achieved.

When the core diameter is reduced to $\phi_{\text{core}} = 100 \, \mu$m, the $z_T$ transition distance is reduced to less than 500 $\mu$m for all cases. This allows the effect of core diameter to be ignored and only the NA investigated. Figure 2.15b shows this reduced core diameter case, with the spot size on the nerve increasing linearly with increasing NA and the temperature showing a $T \propto NA^{-2}$ relationship, as a smaller NA keeps the light more localised.

### 2.4.3 Stimulation Wavelength

As previously discussed, a wide range of different wavelength ranges have been used for INS. Since the dominant factor in INS is conversion of the optical energy into heat through water absorption, the effect of different laser wavelength on the resulting temperature should be considered. Although more strongly absorbed wavelengths will give a higher temperature closer to the fibre, the temperature further away from the fibre may be lower than for less strongly absorbed wavelengths, as much of the energy may be absorbed before the target for the strongly absorbing case.

The temperatures achieved for wavelength of 1800 nm, 1850 nm, 1870 nm and 1900 nm, with absorption coefficients of 0.82 mm$^{-1}$, 0.96 mm$^{-1}$, 2.0 mm$^{-1}$ and 8.0 mm$^{-1}$ respectively, are shown in Figure 2.16. The wavelengths were selected to cover the range most commonly used for INS (1850 – 1870 nm) and to show contrasting absorption levels, as shown in Figure 2.3. The most strongly absorbed wavelength (1900 nm) shows a much higher temperature near the fibre. Further away from the fibre, at distances such as 500 $\mu$m, less strongly absorbed wavelength provide a higher temperature, as much of the energy is absorbed in tissue before that point with strongly absorbed wavelengths.

The 2D temperature profiles shown in Figure 2.16 provide a convenient illustration of the broad differences between wavelengths. A more quantitative approach allows the finer differences between wavelength to be examined. Figure 2.17 shows the temperature increase at a point in the middle of the nerve layer, with the fibre positioned at varying distances from the centre of the nerve region, in the wavelength range 1400 nm to 1900 nm. A distance of 500 $\mu$m between the fibre and nerve region was again selected as it is a common choice in the literature (Izzo et al., 2007c), 250 $\mu$m and 750 $\mu$m were selected as a comparison for when the fibre is moved closer to and further away from the nerves. The wavelength range between 1800 and
2.4. Modelling of Spatial Behaviour

Figure 2.16: Figure showing temperatures achieved due to absorption at wavelengths of 1800 nm, 1850 nm, 1870 nm and 1900 nm. ($\phi_{\text{core}} = 200$ µm, $NA = 0.22$, $E_{\text{pulse}} = 25$ µJ). Note the varying scale for change in temperature.

Figure 2.17: Temperature change in the nerve layer with fibre positions 250 µm, 500 µm and 750 µm away, calculated over the wavelength range 1400 nm to 1900 nm. $\phi_{\text{core}} = 200$ µm, $NA = 0.22$, $E_{\text{pulse}} = 25$ µJ.
1900 nm is shown with an expanded scale, to highlight changes observed in the range commonly used for optical stimulation.

In general, higher levels of water absorption result in a higher temperature change. However, for the highest levels of absorption considered here (for wavelengths above 1870 nm, where $\mu_a > 2 \text{ mm}^{-1}$), the energy absorbed in the nerve layer can be reduced, as much of the light is absorbed by the perilymph and bone before it reaches the nerves. Changing the fibre position causes the temperature change to have a different dependence on wavelength, due to different levels of absorption before reaching the nerve. For the largest spacing ($750 \mu m$) the absorbed energy dose is consistently reduced for high levels of absorption, i.e. around 1450 nm and for wavelengths above 1870 nm.

![Temperature vs Absorption](image)

**Figure 2.18**: Temperature change in the nerve layer with the fibre positioned 250 $\mu m$, 500 $\mu m$ and 750 $\mu m$ away from the centre of the nerve layer for a range of absorption values. The solid lines are a spline fitting curve to the data points calculated from the model. $\varphi_{\text{core}} = 200 \mu m$, $NA = 0.22$, $E_{\text{pulse}} = 25 \mu J$.

The effects of changing the absorption parameter $\mu_a$, which is determined by the wavelength chosen in the stimulation, can be seen more clearly in Figure 2.18. In this figure the x-axis is plotted in terms of $\mu_a$ rather than wavelength, which has a non-linear change in absorption. Again, the temperature change is due to a 25 $\mu J$ laser pulse. When the fibre is positioned further from the nerve layer the temperature change in the nerves is lower, as expected due to energy being absorbed in the perilymph before the nerve. It also shows that when the absorption coefficient ($\mu_a$) is increased (for example due to the wavelength used) a point is reached where the energy absorbed will decrease, as much of the laser energy is absorbed before it reaches the nerve layer. When the fibre is positioned further away, this turning point in the temperature change in the nerve is reached at lower levels of absorption. It should
be noted that the maximum temperature change, for each fibre distance, corresponds well with the optical penetration depth ($z_a = 1/\mu_a$). Maximum temperature change is at $\mu_a = 4.3 \text{ mm}^{-1}$ for 250 $\mu$m giving $z_a = 1/4.3 = 0.232 \mu$m; $\mu_a = 2.0 \text{ mm}^{-1}$ for 500 $\mu$m ($z_a = 0.5 \mu$m) and $\mu_a = 1.4 \text{ mm}^{-1}$ for 750 $\mu$m ($z_a = 0.714 \mu$m). The absorption coefficient that gives the largest temperature change at each distance, corresponds to the penetration depth for that absorption coefficient. This shows that the wavelengths used for different geometries need to be carefully considered to optimise the energy delivered at the target region. This results also provides a useful “rule of thumb” when selecting a wavelength for a particular stimulation geometry.

In the wavelength range most commonly used for optical stimulation (1800 - 1900 nm), these results show that the energy absorbed in the nerve, when the fibre is positioned 500 $\mu$m away from the nerve layer, increases from $\lambda = 1850$ nm to a maximum at $\lambda = 1870$ nm. This may be compared to the experimental results of Izzo et al. (2007c), who varied the wavelength between 1844 and 1873 nm and found that the response reduced at 1860 nm and was minimal at 1873 nm (with the optical fibre emitter positioned 500 $\mu$m from the nerve). There are a number of possibilities that may account for this discrepancy:

- That either the absorption or scatter due to differences in geometry is important and needs to be considered by the model.
- The distance between fibre and target neurons reported by Izzo et al. (2007c) was inaccurate.
- That diode lasers typically have a broad spectral bandwidth of 10 nm to 20 nm (Duke et al., 2012a), which may change the resultant temperature distribution compared to the assumed narrow bandwidth (see below).
- The assumed $\mu_a$ absorption coefficient used in this modelling is incorrect.
- There is another mechanism behind INS in some cases.

**Laser Bandwidth**

The contribution of a broader spectral bandwidth to the resulting change in temperature from stimulation is shown in Figure 2.19. Here, the Monte Carlo model has been modified to give a non-uniform wavelength and a configurable spectral bandwidth. The spread over different wavelengths is even (step function) and is configured to be a delta function, 10 nm or 20 nm. This range of bandwidths covers a worst case scenario and roughly corresponds to the largest bandwidth for lasers discussed in
Chapter 5. With a distance of 500 µm between the fibre and target nerves, the
greatest effect on the resulting temperature is seen in the wavelength range of 1865 –
1875 nm. At 1870 nm the 0 nm bandwidth case shows a peak in the temperature
change of 0.143 ºC, which is ~ 10% higher than the 20 nm bandwidth case. The
20 nm bandwidth case shows a smaller change over the range 1865 – 1875 nm,
relative to the peak observed for a single wavelength. The 10 nm bandwidth shows a
moderate reduction in the peak temperature compared to a laser with a bandwidth
of zero, although the reduction is not as severe as seen with a bandwidth of 20 nm.
Despite real laser sources not having a bandwidth with an even distribution, the step
function used here represents a worst-case scenario. The broad spectral bandwidth
common in high-power diode lasers may contribute to the difference in effectiveness
noted between the Aculight diode laser and Ho:YAG laser sources noted in Duke
et al. (2012a), but these results suggest that it is not an important difference.

![Figure 2.19: Temperature change in the nerve when the laser’s spectral bandwidth has been
set to 0 nm (delta function), 10 nm and 20 nm. \( \phi_{core} = 200 \mu m, \text{NA} = 0.22, \text{E}_{\text{pulse}} = 25 \mu J. \)](image)

**Selective Absorption**

While it is unlikely that single tissue chromophores can significantly enhance light
absorption and hence promote INS (Wells et al., 2007a), it is possible that absorption
in the tissues is higher than the water dominated level we have assumed here. For
example, data for light absorption in water and both oxygenated and deoxygenated
blood from Roggan et al. (1999) shows an increase in the absorption coefficient of 0.5
to 1.5 mm\(^{-1}\) in blood compared to water around a wavelength of 1850 nm. As the
composition of bone and nerve tissues differs considerably from that of perilymph,
which is primarily composed of water and has similar optical properties to water, it
suggests that the light may be more strongly absorbed in the bone and nerve tissue rather than the perilymph. In Figure 2.20, the absorption coefficient in the bone and nerve has been increased by 1 mm\(^{-1}\) to simulate selective absorption, with the other parameters remaining the same as Figure 2.8. The resulting energy absorbed with a fibre distance of 500 µm can be seen as a function of wavelength in Figure 2.21. With selective absorption there is a decrease in energy absorbed from a plateau between 1800 and 1850 nm as the wavelength increases beyond 1860 nm, similar to the results shown by Izzo et al. (2007c). While selective absorption is not necessary for INS, as it is dominated by water absorption (Shapiro et al., 2012), it may enhance the process in specific cases.

![Figure 2.20: Monte Carlo simulation with selective absorption in nerve and bone layers when the optical fibre is positioned 500 µm from the nerves. White vertical lines show the bone layer between 400 and 450 µm and the nerves between 450 and 550 µm (\(\lambda = 1850\) nm, \(\text{NA} = 0.22\), Core diameter = 200 µm, \(n_{\text{photons}} = 1 \times 10^{11}\), pulse energy = 25 µJ).](image)

### 2.4.4 Thermal Gradient

Some research (Wells et al., 2007a) has suggested that a spatial temperature gradient rather than a net increase in temperature is required to stimulate a nerve. Temporal gradients are achieved by use of short laser pulses, but spatial gradients will depend on fibre design and the absorption coefficient of the nerve. Results from the Monte Carlo simulation can also be used to find the temperature gradient in tissue. Figure 2.22 shows the temperature for wavelengths of 1800 nm, 1850 nm, 1870 nm and 1885 nm along the z axis of the fibre (see Figure 2.7 for axis definitions). The more strongly absorbed a wavelength is, the steeper the spatial gradient.

The spatial gradient can be evaluated for different wavelengths as shown in Figure 2.23. Here a fibre was placed 250 µm, 500 µm and 750 µm away from the nerve
Figure 2.21: Temperature change with and without a selective increase in absorption in the bone and nerve over the wavelength range 1800 nm to 1900 nm.

Figure 2.22: Temperature along the z axis for wavelengths of 1800 nm, 1850 nm, 1870 nm and 1885 nm. The most strongly absorbed the wavelength of light is, the faster the decrease in temperature is. \( \delta_{\text{core}} = 200 \ \mu \text{m}, \ \text{NA} = 0.22, \ E_{\text{pulse}} = 25 \ \mu \text{J}. \)
layer and the spatial temperature gradient was taken with respect to the z axis. The gradient is considered in the z axis, as this is determined by the absorption of the particular wavelength, while the x and y axes are dependent on the fibre emitter. Similarly to the peak temperature, when the fibre is closer to the nerve, the gradient in temperature is higher. For 500 µm and 750 µm the gradient reduces when the optical penetration depth drops below the fibre distance, corresponding to wavelengths above ~ 1880 nm.

![Figure 2.23](image)

Figure 2.23: Spatial temperature gradient as a function of wavelength, with the fibre positioned at 250 µm, 500 µm and 750 µm from the middle of the nerve layer. \( \varphi_{\text{core}} = 200 \mu\text{m}, \text{NA} = 0.22, E_{\text{pulse}} = 25 \mu\text{J}. \)

The spatial temperature gradient observed in the modelling is quite small in comparison to the temporal temperature gradient, which would be expected from short laser pulses. Over a spiral ganglion neuronal cell body (diameter \( \approx 10 \mu\text{m} \)), the change in temperature would be between 0.002 °C and 0.018 °C. It is unclear what biophysical response a cell would have to a spatial temperature gradient of this magnitude. It is therefore more likely that the temporal gradient is a factor in successful nerve stimulation. This is supported by the recent results of Shapiro et al. (2012) who use of a model of membrane capacitance to explain the mechanism of INS. In the capacitance model, a redistribution of charge is driven by a time dependant change in temperature. The temporal gradient is discussed in Chapter 3, where a finite element model of heat conduction is introduced.

### 2.4.5 Sciatic Nerve

A comparison between the cochlear model and sciatic nerve model is shown in Figure 2.24, where light emitted from a 200 µm core diameter fibre is targeting
a nerve. The primary difference between the cochlea and sciatic nerve is that scattering occurs throughout the model for the sciatic nerve, rather than only in the thin bone and nerve layers, as in the cochlea. This additional scattering may diffuse the light before it reaches target tissue. The results with standard scattering coefficients \( \mu_s = 1 - 2 \text{ mm}^{-1} \) in Figure 2.24 visually suggest a minimal change in the behaviour of light over these distances. This suggests that the results from the cochlear model are comparable with other geometries and target nerves over short distances \( z < 500 \text{ µm} \).

![Figure 2.24: Examples of Monte Carlo simulations for sciatic nerve and cochlear geometries. \((\lambda = 1850 \text{ nm}, NA = 0.22, \varnothing_{\text{core}} = 200 \text{ µm}, n_{\text{photons}} = 1 \times 10^{11}, E_{\text{pulse}} = 25 \mu J)\).](image)

The difference in temperature achieved can be compared more easily by taking the temperature along the axis of the fibre as shown in Figure 2.25. As the scattering coefficient is assumed to be low, there is only a minimal difference in temperature, compared to the cochlear case, which is mostly comprised of water. A slight initial increase in temperature is observed (due to back scattered light), and the temperature remains similar until it diverges below the cochlea case at \( z \approx 300 \text{ µm} \). A comparison for a higher scattering values of \( \mu_s = 2 \text{ mm}^{-1} \) and \( 5 \text{ mm}^{-1} \) are also provided. With the higher scattering value, the temperature decreases faster, diverging strongly from \( z \approx 200 \text{ µm} \) and displaying a reduced temperature further from the fibre compared
to the other cases.

Figure 2.25: Change in temperature along the z axis for the analytical case with no scattering the cochlear model with $\mu_s = 1 \text{ mm}^{-1}$ and sciatic nerve with $\mu_s = 1 \text{ mm}^{-1}$, $2 \text{ mm}^{-1}$ and $5 \text{ mm}^{-1}$, $\phi_{\text{core}} = 200 \text{ $\mu$m}$, NA = 0.22, $E_{\text{pulse}} = 25 \text{ $\mu$J}$.

The results presented show that the behaviour in bulk neural tissue is expected to be similar to that in water for the parameters assumed (particularly the low $\mu_s$) and the optical fibres used in the experiments. However, as there is generally a tissue-air interface for the sciatic nerve experiments, rather than a tissue-water interface, this may change the temperature when heating effects are considered. Additionally, the differences encountered here are not great enough to explain the order of magnitude difference in radiant exposure thresholds demonstrated in experimental work (Wells et al., 2007a).

### 2.4.6 Cell Culture

Monte Carlo modelling can also provide insight into the change in temperature that cells are exposed to during *in vitro* cell culture stimulation experiments. In these experiments, the fibre is typically close to the target cells. The increased proximity of the fibre reduces the absorbent material, such as water, between the fibre and nerve cell, resulting in a temperature change that is simpler to estimate. Modelling of INS for these experiments may allow a more precise understanding of the spatial variations in temperature that target neurons are exposed to. This may assist in understanding the biophysical mechanisms of INS, a key area of research when using *in vitro* models (Shapiro et al., 2012; Albert et al., 2012).

Figure 2.26 shows an example of the the heat generated when targeting cells located on a glass coverslip, using experimental arrangement shown in Figure 2.6.
In this arrangement, the fibre approaches the target cell on a coverslip at an angle with a micro-pipette targeting the cell from the opposite upper left hand of the figure. It is worth remembering that absorption in glass is negligible compared to water (Table 2.1). Here a wavelength of $\lambda = 1850$ nm is used with a pulse energy of $E_{\text{pulse}} = 330$ $\mu$J, typical of a 1 ms pulse used by Brown et al. (2013). If a pulse energy of 25 $\mu$J is used, the peak temperature achieved near the fibre is the same as that in the cochlea case, although the temperature at the target cells is higher due to the proximity of the fibre to the cells.

Figure 2.26: Example of a Monte Carlo simulation with the cell culture arrangement. $\lambda = 1850$ nm, $\varnothing_{\text{core}} = 200$ $\mu$m, NA = 0.22, $E_{\text{pulse}} = 330$ $\mu$J. The position of the fibre core is shown with white lines, the cladding (220 $\mu$m) is not marked. The white lines at $z = 400$ and 700 $\mu$m show the glass coverslip and the perspex base of the bath.

Figure 2.27: Temperature profile along the x axis of the simulation, with the temperature along the glass coverslip and 10 $\mu$m away from the slide. $\lambda = 1850$ nm, $\varnothing_{\text{core}} = 200$ $\mu$m, NA = 0.22, $E_{\text{pulse}} = 330$ $\mu$J.
2.5 Conclusion

The temperature induced by the laser heating can also be displayed by taking the profile along the glass slide, across the slide in the $y$ direction and with the fibre positioned away from the slide surface. Figure 2.27, shows the temperature profile along the $x$ axis at the centre of the beam, 75 $\mu$m off the fibre axis ($y = 75 \mu$m) and with the fibre positioned 10 $\mu$m away from the slide ($z = 10 \mu$m). In the region directly exposed to the beam, there is only a small variation from a peak temperature of 2.4 °C at the point where the fibre touches the slide to 1.9 °C where the beam begins to expand more. This gives a region of approximately 300 $\mu$m where the temperature does not have any large spatial variation. When the fibre is 75 $\mu$m to the side, this reduces the region with minimal variability. These variations provide an indication of the precision with which the fibre should be positioned with respect to the target cell, in order to achieve a given thermal effect. However, the proximity of non-absorbing mediums close to the heated cells may change the final temperature. The potential for this to reduce the temperature is addressed in Chapter 3.3.1 with the addition of a finite element model to simulate heat conduction.

2.5 Conclusion

Monte Carlo modelling can provide useful insights into the light-tissue interactions that underpin the phenomenon of infrared nerve stimulation. The model presented here gives information on the expected performance of different optical fibre designs and laser sources for light delivery to target tissue. This information could be used to optimise the emitter and implant design. Given that there is an identifiable threshold for all reported instances of INS, it is clear that the underlying mechanism must be driven by an initial absorption process. The model allows the actual absorbed dose of energy, which is therefore a more fundamental quantity than radiant exposure. The temperature dependence can be compared with observed thresholds as a function of wavelength under different assumptions about the underlying absorption process. The results presented suggest that a temperature increase of as little as 0.1 °C may be involved in stimulation of the cochlea.

The results show that the Monte Carlo model is robust within the uncertainty in the parameters and provides comparable results to experimental measurements over the range of parameters used. It also illustrates where more fundamental approximations are still valid, such as the simple analytical case, and where the use of a more advanced model is required to properly understand the resultant temperatures that are generated in tissue.
Modelling of the temporal behaviour of INS

Although the Monte Carlo model presented in Chapter 2 provides information about the spatial distribution of light absorption and the resultant heating, it does not show the change in heat distribution as heat flows away from the irradiated area during exposure and after illumination has ceased. This is especially important for INS, as it is thought to rely on a thermal gradient with respect to time (Shapiro et al., 2012). The importance of movement of heat may be greater in situation where an interface between absorbing and non-absorbing media is present, as there will be a strong spatial thermal gradient, which in turn, promotes a greater temporal gradient. This change between absorbing and non-absorbing media may be especially important in cell culture experiments, where cells are located on glass slides, or peripheral nerve stimulation, where the nerve is often exposed to the atmosphere.

This chapter details the development of a finite element solution to the heat equation and other factors that may need to be considered when studying the change in temperature over time during INS. Section 3.1 introduces the heat equation and the development of a finite element model (FEM) that solves it. Validation of the thermal model, through comparison with published experiment results, is shown in Section 3.2. Section 3.3 investigates the influence of heat conduction on
Chapter 3. Modelling of the temporal behaviour of INS

the temperature resulting from millisecond duration pulses. Section 3.4 looks at how differing fibre geometry changes thermal decay times. Finally, the thermal model is applied to microsecond duration pulses in Section 3.5, to investigate the differences between some key cochlea stimulation experiments and to determine whether modelling can provide further insights into the thermal requirements of INS.

Portions of this chapter have previously been published in the following publications (Thompson et al., 2013b,c).

3.1 Heat Transfer

When different parts of a body are at different temperatures, heat flows from the hotter to cooler parts. There are three distinct mechanisms by which this can occur: heat conduction, where the heat is transmitted by the vibrational energy of atoms being passed on; convection, where heat is transmitted by the motion of a heated gas or liquid to a cooler region; and radiation, where heat is transmitted directly by electromagnetic radiation (Carslaw, 1986). In the case of laser-tissue interactions, usually only heat conduction needs to be considered. Convection is usually not considered in heat transfer during laser tissue interactions, partly due to the low perfusivity of most tissues (Niemz, 2007) and the short interaction time used. However, it is worth considering the impact of convective processes on final temperatures, especially in cases where tissue is exposed to air or liquid, or when long stimulation durations are used (> 1 second). There are a number of processes through which convective heat transfer can occur during INS, some of which are general to all target neurons and others that are specific to particular targets. These effects are considered in the next Chapter (Section 4.3), where multiple pulse trains over longer periods are considered. As radiative heat transfer depends on temperature to the fourth power ($T^4$, Stefan-Boltzmann law) (Niemz, 2007), its contribution is usually considered to be negligible at or near room temperature.

3.1.1 Heat Conduction

Heat conduction in a homogeneous, isotropic solid is described by the heat equation, a partial differential equation (PDE):

$$\frac{\partial u}{\partial t} = \alpha \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} \right),$$

(Moin, 2010). The thermal diffusivity ($\alpha$) describes the rate at which a material changes temperature and can be thought to describe a material’s thermal inertia. A
3.1. Heat Transfer

Material with higher thermal diffusivity allows heat to spread out faster and reaches an equilibrium faster. Thermal diffusivity is given by:

$$\alpha = \frac{k}{\rho c_p},$$

(3.2)

where $k$ is the thermal conductivity, $\rho$ the density and $c_p$ the specific heat capacity of the material.

To describe the rate at which heat is added to the material by absorption of light from a laser, a new term $A$ can be introduced:

$$\frac{\partial u}{\partial t} = \alpha \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2}\right) + A(t).$$

(3.3)

General analytical solutions of the heat equation are possible for simple geometries (Carslaw, 1986; Pennes, 1948; Unsworth, 1979; Halldórsson and Langerholc, 1978), but in more complex geometries a finite element modelling (FEM) approach is generally more straightforward (Moin, 2010). Solutions for the heat equation have been used to better understand light-tissue interactions in processes such as laser ablation (Chen et al., 2006) and heating during infrared spectroscopy (Ito et al., 2000).

A general analytical solution of the heat equation gives the thermal penetration depth (Niemz, 2007):

$$\delta_z = \sqrt{4\alpha t},$$

(3.4)

which describes the spatial extent of heat transfer during time $t$. As this relationship is derived from the exponential part of the one-dimensional Green’s function solution, it gives the distance where the temperature has decreased to $1/e$ of its peak value (Niemz, 2007). The thermal penetration depth can be re-arranged to give the thermal relaxation time for a specific heated region. The thermal relaxation time, gives the time taken for the temperature to fall to $1/e$ of the original value:

$$t = \frac{\delta_z^2}{4\alpha}.$$  

(3.5)

If the optical penetration depth ($z_a = 1/\mu_a$) is then taken as a measure of the heated region, we can then find the characteristic thermal relaxation time for different wavelengths:

$$\tau_r = \frac{z_a^2}{4\alpha}.$$ 

(3.6)

The thermal relaxation time for the wavelength range 1400 – 2500 nm in a material with the thermal diffusivity of water ($\alpha = 1.43 \times 10^{-7} \text{ m}^2\text{s}^{-1}$) is shown in Figure 3.1, with the commonly used wavelengths 1850, 1870 and 2120 nm highlighted.
At 1850 nm, the relaxation time is 2.0 seconds, while the more strongly absorbed wavelengths of 1870 nm and 2120 nm have relaxation times of 0.49 s and 0.34 s respectively. While this allows for simple comparison of the absorption and thermal relaxation characteristics of different wavelengths, when the diameter of the irradiated area is equal to or less than the optical penetration depth, heat diffusion in the radial direction needs to be taken into account as well. Therefore, the estimated thermal relaxation time does not take into account the effect of different sized irradiation areas, such as may arise due to variations in fibre core diameter and NA.

![Diagram showing thermal relaxation time over the 1400 – 2500 nm range, \(\mu_a\) absorption values for comparison. The absorption range of 1 – 2 mm\(^{-1}\) is highlighted to show the absorption range most favourable for INS and commonly used INS wavelengths of 1850, 1870 and 2120 nm highlighted.]

### 3.1.2 Finite Element Solution

To allow the heat equation to be solved for more complex geometries, a finite element solution was developed. A range of techniques exist to solve parabolic partial differential equations, such as the heat equation, with different stability requirements, accuracy and easy of implementation. The simplest is the forward-difference method, a conditionally-stable method (Burden and Faires, 1985) with a stability criterion for the heat equation of

\[
\frac{1}{2} \leq \alpha d \frac{\Delta t}{x^2},
\]

where \(\Delta t\) is the timestep of the simulation, \(x\) is the size of each voxel or distance between elements, \(\alpha\) is the thermal diffusivity and \(d\) is the number of dimensions the simulation is being run over. This can be rearranged to find the maximum stable
timestep when running a simulation in 3 dimensions

\[ \Delta t_{\text{max}} = \frac{x^2}{6\alpha}. \]  \hspace{1cm} (3.8)

When using a voxel size of 5 \(\mu m\) in water, this gives a maximum time step of 29 \(\mu s\), meaning a simulation of duration \(t_{\text{len}} = 100\) ms would take a minimum of 3449 steps. If a smaller thermal diffusivity (\(\alpha\)) is used, a smaller time step must be used, therefore computation will take a longer time to complete. The time step used must also be smaller than the laser pulse length.

Other difference method solutions, such as the backward-difference method and Crank-Nicolson method are unconditionally-stable and do not have any requirements for stability (Burden and Faires, 1985). However, they are more numerically complex to solve and given the strong computational abilities of modern computers are only advantageous for this work when the choice of parameters would force the timestep to very small values or long times are required to be simulated. Given a voxel size of 5 \(\mu m\) is adequate and on order of the size of target neurons, the forward-difference method was selected for this work.

The simulation was implemented in OpenCL, a hardware-agnostic application programming interface (API) for general purpose computing on CPUs and GPUs. Central Processing Units (CPUs) are the general computation in computers, modern CPUs have good performance and are flexible. Many computers additionally have a Graphics Processing Unit (GPU), although designed for rendering computer graphics, modern GPUs are also able to perform highly parallel computational tasks. This approach takes advantage of the high performance computing power of modern CPUs and GPUs to reduce computation time (Owens et al., 2008; Goddeke et al., 2009; Thompson et al., 2010). The simulation was written to target Intel's CPU OpenCL Software Development Kit (SDK), as it did not require the same time investment in optimising memory access as is required when using GPU targets (Thompson et al., 2010) and performance was found to be adequate on an Intel Xeon W3520\(^1\).

The simulation loads data saved from the Monte Carlo simulation presented in Chapter 2 to provide the spatial distribution of instantaneous temperature increase due to light absorption. Unlike the Monte Carlo model, a 5 \(\mu m\) voxel size is used here to reduce computation time (total voxel count reduced to 1/8) and to reduce memory requirements. The simulation runs over a 258 \(\times\) 258 \(\times\) 258 voxel 3D grid, with the central 256 \(\times\) 256 \(\times\) 256 voxel actually having computation performed on

\(^1\)The Intel Xeon W3520 has 4 cores (8 virtual cores when using hyperthreading) running at 2.67 GHz; using OpenCL gave a \(\sim 6\times\) performance increase compared to a standard OpenMP C++ implementation.
them. A size of 256 was selected to allow easy division by factors of $2^x$ which can be important for optimising OpenCL targets. The initial heat distribution is provided in a $200 \times 200 \times 200$ 3D grid from the Monte Carlo simulation. A flow diagram of the simulation is shown in Figure 3.2.

![Flow diagram showing the operation of the finite element model.](image)

After initialising the required data structures, the stimulation begins. A fraction of pulse data is added to the current state, corresponding to the timestep over total pulse length ($\Delta t/t_{pulse}$), the heat flow for each voxel is calculated

$$\Delta T(x,y,z) = T(x+1,y,z) + T(x-1,y,z) + T(x,y+1,z) + T(x,y-1,z) + T(x,y,z+1) + T(x,y,z-1) - 6T(x,y,z),$$

(3.9)

and then the current state is updated by taking the time step ($\Delta t$) and thermal diffusivity of that position ($\alpha(x,y,z)$) into account

$$T_{(x,y,z)} = T_{(x,y,z)} + \frac{\Delta t \alpha(x,y,z)}{\Delta x^2} \Delta T_{(x,y,z)},$$

(3.10)
As the temperature during the simulation and not just at the completion is of interest, a 2D slice from the x-z plane in the middle of the y-axis of the stimulation is saved at a configurable time interval. Only one 2D slice is saved, as the x and y axes are typically symmetrical and it significantly reduces the hard disk storage required\(^2\). Once the simulation is complete, a full 3D representation of the data is saved. Only the middle \(200 \times 200 \times 200\) 3D grid from the data is saved, corresponding to the area that had the input data from the Monte Carlo simulation.

### 3.1.3 Parameters used for Simulations

The only parameter required for the finite element model is the thermal diffusivity of the medium. Here the thermal diffusivity is presented for the various media used, as shown in Table 3.1. The thermal diffusivity of water \((\alpha = 1.43 \times 10^{-7} \text{ m}^2\text{s}^{-1})\) was used for tissue and perilymph, as reductions in thermal conductivity due to a reduced water content are usually compensated by a similar reduction in heat capacity (Boulnois, 1986). The thermal diffusivity of fused quartz was taken as \(8.3 \times 10^{-7} \text{ m}^2\text{s}^{-1}\) (Bergman and Incropera, 2011) and \(3.7 \times 10^{-7} \text{ m}^2\text{s}^{-1}\) for generic glass. For thermal diffusivity of air at room temperature, a value of \(2.0 \times 10^{-5} \text{ m}^2\text{s}^{-1}\) was used (Bergman and Incropera, 2011).

<table>
<thead>
<tr>
<th>Media</th>
<th>Thermal Diffusivity (\alpha) ((\text{m}^2\text{s}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>(1.43 \times 10^{-7})</td>
</tr>
<tr>
<td>Perilymph</td>
<td>(1.43 \times 10^{-7})</td>
</tr>
<tr>
<td>Bone</td>
<td>(1.43 \times 10^{-7})</td>
</tr>
<tr>
<td>Nerve</td>
<td>(1.43 \times 10^{-7})</td>
</tr>
<tr>
<td>Fused Quartz</td>
<td>(8.3 \times 10^{-7})</td>
</tr>
<tr>
<td>Glass</td>
<td>(3.7 \times 10^{-7})</td>
</tr>
<tr>
<td>Air</td>
<td>(2.0 \times 10^{-5})</td>
</tr>
</tbody>
</table>

Table 3.1: Thermal diffusivity used for different media in simulations.

### 3.1.4 Sensitivity Analysis of Boundary Conditions

The original Monte Carlo temperature data was a \(200^3\) grid and the finite element simulation of temporal behaviour runs over a \(256^3\) grid. A simple sensitivity analysis was performed to evaluate what effect this buffer between the Monte Carlo input and the edge of the finite element simulation has and if a more detailed boundary condition is required.

\(^2\)Saving a 2D slice reduces the storage required by a factor of 200. A data file with 100 points requires \(\sim 13\) MB when using gzip compression, if saved in 3D this would require \(\sim 3.4\) GB.
The current arrangement gives buffers of 28 voxels or 140 $\mu$m on each edge of the simulation. Beyond the $256^3$ grid the temperature was held constant at a reference temperature of 21 °C (room temperature). As this boundary condition could provide an artificially high rate of temperature decay, a comparison was made against a 780 $\mu$m buffer or 156 voxels. Using a 1 ms pulse of 1850 nm light from a 200 $\mu$m core fibre, the temperature at a point 500 $\mu$m from the fibre rises to 3.63 °C at the end of the pulse in both cases. After $t = 300$ ms, the temperature for the small buffer case was 1% lower than the 780 $\mu$m buffer. By 500 ms the temperature with the small was 6% lower at a temperature of 0.121 °C compared to 0.129 °C for the 780 $\mu$m buffer. This suggests that the buffer size is not a significant factor for $t \lesssim 300$ ms and that the boundary condition used is adequate.

3.2 Model validation against experimental work

To check the accuracy of the model and provide further information about the temporal behaviour of heating during INS, the geometry for the model was arranged to match the experimental arrangement used by Shapiro et al. (2012) for oocyte stimulation. This experimental arrangement used by Shapiro et al. (2012) provides temperature measurements with good precision ($< 0.1$ °C) and spatial accuracy to within 100 $\mu$m. Oocyte stimulation was performed with a 1889 nm laser coupled to a 400 $\mu$m core fibre. During stimulation, the fibre was positioned below a 100 $\mu$m thick quartz coverslip and aimed up towards the oocyte. When performing temperature measurements, the oocyte was removed and temperature measurements were made using pipette resistance with the pipette positioned in the centre of the beam, within 100 $\mu$m of the coverslip. Peak temperature changes of 15.2 °C and 27.2 °C were observed for pulses of (2.8 mJ) 1 ms and (5.6 mJ) 2 ms, respectively. The model was configured with the fibre positioned 500 $\mu$m behind the 100 $\mu$m quartz coverslip, aimed at the oocyte on the other side. The oocyte was given the same optical and thermal properties as water for simplicity. As the temperatures (Shapiro et al., 2012) were measured within 100 $\mu$m of the coverslip along the centre of the fibre, readings presented here were taken over a range from the coverslip surface for both pulse lengths considered.

Figure 3.3(a) shows the change in temperature at positions 0, 25, 50 and 100 $\mu$m above the glass surface for a 1 ms pulse. The peak temperatures are summarised in Table 3.2. The cases where the temperature is measured at 50 $\mu$m or closer to the glass slide initially show a more rapid decrease in temperature than the 100 $\mu$m case, which displays a slight increase in temperature for $\sim 5$ ms after the pulse. The shape
3.2. MODEL VALIDATION AGAINST EXPERIMENTAL WORK

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Modelled Result</th>
<th>Measured Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ms, 0 µm</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>1 ms, 25 µm</td>
<td>20.7</td>
<td></td>
</tr>
<tr>
<td>1 ms, 50 µm</td>
<td>18.3</td>
<td>15.2</td>
</tr>
<tr>
<td>1 ms, 100 µm</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>2 ms, 0 µm</td>
<td>30.5</td>
<td></td>
</tr>
<tr>
<td>2 ms, 25 µm</td>
<td>39.9</td>
<td></td>
</tr>
<tr>
<td>2 ms, 50 µm</td>
<td>36.5</td>
<td>27.2</td>
</tr>
<tr>
<td>2 ms, 100 µm</td>
<td>27.8</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2: Summary of simulated temperatures compared with results from Shapiro et al. (2012). The parameters indicate the laser pulse length and distance of the measurement from the coverslip.

Figure 3.3: Modelled change in temperature from an INS pulse and during the resulting decay using the geometry of Shapiro et al. (2012).
of the experimentally measured temperature decay and increase from (Shapiro et al., 2012) shown in Figure 3.4 closely matches that calculated when the temperature is taken within 50 µm of the coverslip. The exact position of the micropipette was uncertain and only known to be within 100 µm of the coverslip. Given the experimental uncertainty in this measurement, the model closely matches the result observed experimentally. The temperature gradient displayed in Figure 3.3(b) also shows a similar trend, with a ∼ 25% reduction in the gradient over the first 200 µs of the pulse, when the temperature is measured on the glass. The model suggests that moving the fibre so it is touching the coverslip increases the temperatures by 20% to 30% due to a reduction in the spread of the beam. The model compares well with the experimental measurements of temperature and shows a decrease in the ratio of temperature to pulse energy similar to that observed in (Shapiro et al., 2012).

### 3.3 Millisecond Duration Pulses

Due to the thermal diffusivity of water and tissue, it is reasonable to expect that a significant amount of heat conduction will occur during millisecond duration pulses. This may affect the resulting peak temperature and temperature distribution.

To assess the effect of heat conduction on pulses of various length, the output from a continuous wave laser is considered. Figure 3.5 shows (a) the temperature and (b) the instantaneous rate of change of temperature (henceforth referred to as the temperature gradient) when the laser is turned on at $t = 0$. These results were based
on a 10 mW laser beam with wavelengths of 1850 nm and 1870 nm, measured in a nerve layer 500 µm from an optical fibre with 200 µm core diameter and 0.22 NA. For 1850 nm, the temperature has increased by 0.36 °C, after 10 ms, compared to 0.45 °C for no conduction, a reduction of 20%. The temperature gradient shows an even greater reduction, falling from an initial value of 0.045°C.ms⁻¹ to 0.030°C.ms⁻¹ by 10 ms, a change of 35%. A wavelength of 1870 nm shows similar trends to the 1850 nm. Therefore, for millisecond duration pulses, thermal conductivity has a significant effect on the final temperature and estimations of the temperature rise due to an instantaneous pulse are not representative of the actual temperature. Extrapolating from the results, the temperature may reach equilibrium after ∼500 ms, where any further heat added by the laser is conducted away at the same rate.

![Figure 3.5](image)

**Figure 3.5:** (a) The temperature rise generated by a 10 mW laser running CW over 100 ms: solid lines show the temperature with conduction taken into account; dashed lines show the temperature if there was no conduction. (b) Temperature gradient from a 10 mW laser running CW over 100 ms.

While short laser pulses \((t < 100 \mu s)\) have been shown to be most effective for INS in the cochlea, there are also many examples in the literature of millisecond duration pulses being used for INS (Wells et al., 2007a; Shapiro et al., 2012; Albert et al., 2012). As in Chapter 2, water is used as both the transmission, absorption, scatter and conduction medium as it has similar properties to tissue (Boulnois, 1986) and simplifies comparisons between different wavelengths and distances from the optical fibre to the target position. For a 1 ms pulse at \(\lambda = 1850\) nm, the change of temperature over time is illustrated in Figure 3.6, showing snapshots at 0.5 ms, 1 ms, 10 ms and 20 ms. For \(\lambda = 1850\) nm, the temperature is reported 500 µm from the fibre, while for \(\lambda = 1937\) nm the temperature is taken 55 µm from the fibre. The different distances are due to the significantly higher absorption for \(\lambda = 1937\) nm.
compared to 1850 nm and corresponds to a distance between the fibre and nerve used in experimental work (Izzo et al., 2008b). For purposes of comparison, 1870 nm is provided at both distances. Figure 3.7 shows the normalised change in temperature from a single pulse of 1 ms, with various wavelengths.

Figure 3.6: Change in temperature from a 1 ms pulse ($\lambda = 1850$ nm, $E_{\text{pulse}} = 25$ $\mu$J) at 0.5 ms 1 ms, 10 ms and 20 ms.

Figure 3.7a shows the temperature profile, with the full 1 ms pulse and first 4 ms of thermal evolution highlighted in the insert, using wavelengths of 1850 nm, 1870 nm and 1937 nm with pulse energies ($E_{\text{pulse}}$) selected to give the same peak temperature. With excitation 500 $\mu$m away from the nerve, both 1850 nm and 1870 nm display an immediate decrease in temperature, however, when the excitation is shifted to just 55 $\mu$m from the nerve the 1870 nm has a slower decay in temperature while the 1937 nm pulse displays an increase in temperature for around 1 ms after the pulse has finished and before starting to decay. Over longer time periods, both wavelengths at 500 $\mu$m from the fibre display similar decay rates. At 55 $\mu$m, the decay is initially slower than that of 500 $\mu$m, but after 10 ms it overtakes the temperature decay at 500 $\mu$m. The 1937 nm pulse also has a more rapid decay than 1870 nm. The faster decay to baseline temperature with more strongly absorbing wavelengths is expected as the heating is more localised at the fibre tip (Wells et al., 2007a; Izzo et al., 2007a).
3.3. Millisecond Duration Pulses

Figure 3.7: Change in temperature from a 1 ms pulse. a) To allow for easier comparison, pulse energies have been scaled to give the same temperature increase. 1850 nm: \( E_{\text{pulse}} = 25 \, \mu\text{J} \); 1870 nm: \( E_{\text{pulse}} = 20.27 \, \mu\text{J} \) (at 500 \( \mu\text{m} \)); 1870 nm: \( E_{\text{pulse}} = 8.23 \, \mu\text{J} \) (at 55 \( \mu\text{m} \)) and 1937 nm: \( E_{\text{pulse}} = 2.43 \, \mu\text{J} \). b) All pulse energies have been kept constant at 25 \( \mu\text{J} \). Temperatures achieved are: 1850 nm : \( T = 0.11^\circ\text{C} \) (at 500 \( \mu\text{m} \)); 1870 nm : \( T = 0.14^\circ\text{C} \) (at 500 \( \mu\text{m} \)); 1870 nm : \( T = 0.34^\circ\text{C} \) (at 55 \( \mu\text{m} \)) and 1870 nm : \( T = 1.16^\circ\text{C} \) (at 55 \( \mu\text{m} \)).

Figure 3.7 b shows when a pulse energy of 25 \( \mu\text{J} \) is used for all wavelengths. A temperature of 0.11°C is achieved for 1850 nm at 500 \( \mu\text{m} \), 0.14°C for 1870 nm at 500 \( \mu\text{m} \), 0.34°C for 1870 nm at 55 \( \mu\text{m} \) and 1.16°C for 1937 nm at 55 \( \mu\text{m} \). During temperature decay, the pulses with the higher initial temperature remain higher than the other pulses, even 250 ms after the pulse.

It is also worth noting that the spatial localisation of light, driven by the fibre parameters and absorption coefficient at the wavelength selected, is the most significant factor driving changes in thermal relaxation times. At 500 \( \mu\text{m} \) from the fibre with a wavelength of 1850 nm and 1870 nm, there is minimal difference in the temperature after 250 \( \mu\text{s} \), while at 55 \( \mu\text{m} \) there is a larger difference between the two wavelengths (1870 nm and 1937 nm). It is interesting that the thermal relaxation time for the wavelengths is not a good predictor of thermal decay times at different positions. Thermal relaxation times are calculated to be 2.021 s, 489 ms and 13 ms for the wavelengths of 1850 nm, 1870 nm and 1937 nm respectively. However, at 55 \( \mu\text{m} \) the decay time (time to return to 1/\( e \) of peak temperature after pulse has finished) is 23.2 ms and 20.1 ms for wavelengths of 1870 nm and 1937 nm respectively, while at 500 \( \mu\text{m} \) decay times of 33.1 ms and 32.1 ms are seen for wavelengths of 1850 nm and 1870 nm respectively. It is clear from these results, that the additional spatial confinement for the less strongly absorbed wavelengths allows for significantly faster thermal decay. For the 1937 nm case, it is likely that the peak temperature, which
Decay time (ms)

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>1850 nm</th>
<th>1870 nm</th>
<th>1937 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical</td>
<td>2021</td>
<td>489</td>
<td>13</td>
</tr>
<tr>
<td>55 µm</td>
<td>-</td>
<td>23.2</td>
<td>20.1</td>
</tr>
<tr>
<td>500 µm</td>
<td>33.1</td>
<td>32.1</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.3: Summary of thermal decay times for wavelengths shown in Figure 3.7.

will be closer to the surface due to the strong thermal gradient with such a strongly absorbed wavelength, will return to $1/e$ faster. However, this discussion shows the limitations in using the thermal relaxation time based upon just the absorption coefficient of the wavelength and the importance of confining the light to just the area requiring stimulation.

To test the thermal decay behaviour of heated tissue, the model was modified to heat a point instantaneously. The resultant decay curve for this heating is shown in Figure 3.8. If a single exponential curve ($T(t) = A \exp(-Bt)$) is fitted to the decay, it does not agree well with the model results and returns to the baseline level faster than predicted. A sum of exponentials ($T(t) = A \exp(-Bt) + C \exp(-Dt)$) provides a closer fit than a single exponential, closely matching the decay. While a sum of exponentials is convenient function to fit to the data, it is not clear if it has physical meaning. To gain further insight into the thermal behaviour, an analytical solution for the temperature decay on-axis from a collimated beam of light in a material of uniform absorption ($\mu_a$) and thermal diffusivity ($\alpha$) was developed (Cadusch, 2012). This solution suggests that as $t \to \infty$, the decay is best represented by $T(t) \propto t^{-3/2}$ which has a longer decay time than either an exponential or sum of two exponentials. This slower decay may limit the frequency of repeated pulses that can be used before the tissue heats beyond a damage inducing threshold.

### 3.3.1 Effect of non-absorbing media

For most applications of INS the nerves are surrounded by tissue that absorb light in a similar manner to the nerves, which implies that there is no strong spatial temperature gradient and any gradient present is simply due to the absorption of light or beam shape. However, cell culture experiments use explanted neurons on a glass surface which does not strongly absorb light and thus creates a strong spatial temperature gradient. As these experiments are typically aimed at unravelling the biophysical mechanisms behind INS (Shapiro et al., 2012; Albert et al., 2012; Brown et al., 2013), differences in heat flow in these in vitro experiments compared to the in vivo situation may be important to obtain a more complete understanding of the
3.3. **Millisecond Duration Pulses**

**Figure 3.8:** Thermal decay after heating a point instantaneously, with single exponential and sum of exponential fits. Shown with a linear scale on the left and log-log scale on the right.

The mechanisms of INS. The effect of the glass substrate on temperature can be examined by using the geometry of Shapiro et al. (2012), previously discussed in Section 3.2.

**Figure 3.9:** Change in temperature at end of 1 ms and 2 ms pulses and with no heat conduction considered compared to distance from a non-absorbing glass slide. $\lambda = 1889$ nm, $E_{\text{pulse}} = 2.8 \, \mu$J.

Figure 3.9 shows the temperature distribution as a function of distance above the glass surface at the end of 1 ms and 2 ms pulses of the same energy ($E_{\text{pulse}} = 2.8$ mJ). The results are compared with the temperature distribution if heat conduction is ignored. When conduction effects are included, there is substantial difference in temperature at the interface of the glass coverslip. When conduction is not considered, the temperature changes by 24.1$^\circ$C, while the 1 ms and 2 ms pulses cause changes...
of 16.2°C and 15.3°C respectively. Beyond the $\delta_z$ distance (1 ms = 23.9 $\mu$m, 2 ms = 33.8 $\mu$m), the pulses with heat conduction agree reasonably well with the no conduction case. The heat conduction clearly shows the effect of locating cells on a glass slide which is not strongly absorbing at the wavelength of light used for stimulation. This may have a more significant effect on smaller cells, such as spiral ganglion neurons, which are typically 10 $\mu$m in diameter (Needham et al., 2012). The difference is likely to be greater for longer pulses, such as those used by Albert et al. (2012), who used 7 - 10 ms duration pulses. The reduction in temperature in close proximity to the glass slide may not have been measured previously measured, as it is only observed within 20 $\mu$m of the glass slide.

This result highlights the difference in peak temperature when cells are located on a non-absorbing medium and may contribute to some of the differences seen between in vivo and in vitro work.

### 3.4 Effect of Fibre Properties

As discussed in the previous Section 3.3, the characteristic thermal decay time for each wavelength, $\tau_{\text{therm}} = \frac{1}{4 \alpha a^2}$, is not a good predictor of decay time when the light is constrained to a diameter smaller than the optical penetration depth ($1/\mu_a$). The thermal decay time is an important parameter, as it relates to the maximum repetition rate that can be achieved without causing thermal build up (Izzo et al., 2008b). To investigate the effect of varying the fibre core diameter, a range of core diameters between 50 $\mu$m and 400 $\mu$m with either a fixed NA of 0.22 or an adjusted NA to keep $z_T = 600 \mu$m were modelled with a 1 ms duration pulse at a wavelength of 1850 nm. The cochlear model was used, with the fibre located 500 $\mu$m from the centre of the nerve layer. The temperature was taken from the middle of the nerve layer. The thermal decay time ($\tau_{\text{therm}}$) in Figure 3.10 describes the time taken to reduce to $1/e$ of the peak temperature observed in the model. From inspection there appears to be a quadratic dependence between the modelled decay time and the core diameter, although this is masked by the use of a log-linear plot in Fig 3.10.

For the core diameters examined (50 $\mu$m – 400 $\mu$m), light is now more constrained by the fibre than by the optical penetration depth of the wavelength used $z_a = 1/\mu_a = 1042 \mu$m. As the optical penetration depth is the distance constraint used to calculate the thermal decay time for the absorption constrained case ($\tau_r = \frac{z_a^2}{4\alpha}$), an analogous relationship may exist when the core diameter is used as a constraint instead. To test this, a least squares fit was applied with the form $\tau_{r-\text{core}} = \frac{\sigma_T^2}{\lambda \alpha}$, when using the data for a constant $z_T = 600 \mu$m transition distance. A best fit value
of \( A = 8.63 \) was found, and the resultant plot is shown in Figure 3.10.

![Figure 3.10](image)

Figure 3.10: Thermal relaxation time for different fibre core diameters, with \( NA = 0.22 \) or \( z_T = 600 \) µm.

When the NA is kept constant at \( NA = 0.22 \), a similar \( \varphi^2 \) dependence is observed for \( z_T = 600 \) µm. However, with core diameters below 200 µm the decay time remains in the range of 20 – 30 ms. This is due to the greater spread of light for these core diameter and NA combinations.

![Figure 3.11](image)

Figure 3.11: Thermal decay time for different numerical apertures, with \( \varphi_{core} = 100 \) µm, 200 µm and 400 µm. \( z_T \) transition points for 100 µm, 200 µm and 400 µm core diameter fibres are NAs of 0.132, 0.261 and 0.494 respectively.

Figure 3.11 shows the change in relaxation time across a numerical aperture range of 0.11 – 0.44, with fibre core diameters of 100 µm, 200 µm and 400 µm. The behaviour of the decay time diverges from a \( \varphi^2 \) dependence when the combination of the NA and fibre diameter results in the transition point being less than the target...
of stimulation, in this case 500 µm. The 100 µm core fibre, and 200 µm core fibre (when NA > 0.26) give a transition point before the target of stimulation. In this parameter space (z_T < 500 µm) the decay time depends on NA, with a lower NA giving faster thermal decay. The decay time is proportional to the expansion of the beam due to the NA. To develop a fit, a similar analogy can be drawn with the spot size due to expansion as with the core diameter previously. The spot size \( \varnothing_{\text{spot}} \) of the beam at distance \( z \) from the fibre, in a material of refractive index \( n \) is given by \( \varnothing_{\text{spot}} = 2\tan(\sin(\frac{NA}{\pi})))z \). Using the spot size as the distance constraint, a fit can be developed in the form: \( \tau_{\text{thermNA}} = \frac{\varnothing_{\text{spot}}^2}{A\alpha} \). Fitting against the thermal decay time for \( \varnothing_{\text{core}} = 100 \mu m \), in which case the \( z_T \) distance is less than 500 µm for NA > 0.12, gives a value of A = 7.8.

Conversely, when the transition point is beyond the target, there is no strong relationship between the NA and decay time, with only a slight decrease in the decay time towards larger NAs. The 400 µm core fibre and 200 µm core fibre (when NA < 0.26) both illustrate this relationship, showing a thermal decay time characteristic of the respective core diameter (Fig. 3.10).

The spatial localisation of light also varies along the axis of the fibre. Close to the emitter/fibre it is similar to a cylinder, at the transition point and beyond is changes to a cone like shape. Figure 3.12 shows the change in thermal decay time for a 200 µm diameter core fibre with NA = 0.22 along the z-axis. For 200 µm \( \lesssim z \lesssim 600 \mu m \) the decay time is in the range of 32 – 37 ms. Closer to the fibre (\( z < 200 \mu m \)) this reduces due to the unheated fibre and perilymph acting as a heat sink. When \( z \) is greater than the transition point for this fibre and NA combination (\( z_T \gtrsim 600 \mu m \)) the decay time increases, due to the larger spread of the beam.
These results show that restricting the area irradiated during INS allows for faster thermal relaxation times, both through the use of a smaller core diameter and keeping the transition point beyond the target neurons. Minimising the thermal decay time is likely to be important when pulse trains are being used, especially at high pulse rates.

3.5 Microsecond Duration Pulses

Heat conduction during sub-millisecond pulses is likely to have negligible effect on INS and the temperature gradient during the pulse, as the thermal penetration depth ($\delta_z$) is smaller than the laser beam spot size typically used to irradiate the nerves. Nevertheless, it is still useful to confirm the heating properties of pulses under 1 ms and to consider the thermal decay from different pulse lengths. It also allows results to be compared between experiments more easily and also against theoretical calculations.

A comparison can be made between pulses with a fixed energy but variable duration (i.e. laser power is proportional to pulse duration) and a fixed power (i.e. energy is dependent on pulse length). Figure 3.13(a) shows the temperature change during pulses of 35 µs, 100 µs, 350 µs and 1 ms with a constant energy of 2 µJ ($\lambda = 1850$ nm, $d_{\text{fibre}} = 500$ µm, $\mathcal{A}_{\text{core}} = 200$ µm, NA = 0.22), while Fig. 3.13(b) shows the temperature gradient during the same pulses. Figure 3.14 shows pulses using the same parameters as Fig. 3.13 (35 µs, 100 µs, 350 µs and 1 ms) but with a constant laser power of 20 mW for the duration of the pulse: Fig. 3.14(a) displays temperature and (b) temperature gradient. The pulse energy and power were selected to provide representative levels of the thresholds for 100 µs duration pulses with wavelengths and geometry similar to that in the literature. Figure 3.13(a) shows that the increase in temperature is slightly less for longer pulses with the same total energy, due to heat dissipation during the laser pulse. This is confirmed by the temperature gradient for the constant power case (Fig. 3.14(b)), which shows that the gradient decreases slightly over the duration of the 1 ms pulse. Additionally, the decay of heat after the pulse, is over a much greater timescale than the increase of heat from the laser pulse. After the 2 µJ pulse, the temperature gradient reduces to $\sim -0.5$ °C.s$^{-1}$.

The maximum temperature and average temperature gradient can be more easily compared when plotted against the pulse length. The curves in Figure 3.15 show the peak temperature and temperature gradient with different pulse lengths resulting from constant energy pulses (2 µJ) and constant power pulses (20 mW and 100 mW).
Figure 3.13: a) Change in temperature during 35 $\mu$s, 100 $\mu$s, 350 $\mu$s and 1 ms pulses, all with an energy of 2 $\mu$J. b) Temperature gradient during 2 $\mu$J pulses. The inset in b) highlights the thermal gradient during relaxation, note the different scale.

Figure 3.14: a) Change in temperature during 35 $\mu$s, 100 $\mu$s, 350 $\mu$s and 1 ms pulses, all with laser power of 20 mW. b) Temperature gradient during 20 mW pulses.
3.5. Microsecond Duration Pulses

<table>
<thead>
<tr>
<th>Pulse Length (µs)</th>
<th>Radiant Exposure (mJ cm⁻²)</th>
<th>Energy (µJ)</th>
<th>Temperature (°C)</th>
<th>Temperature Gradient (°C ms⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>5.29 ± 0.6</td>
<td>1.66</td>
<td>0.00754</td>
<td>0.216</td>
</tr>
<tr>
<td>100</td>
<td>6.18 ± 1.5</td>
<td>1.94</td>
<td>0.00881</td>
<td>0.0881</td>
</tr>
<tr>
<td>300</td>
<td>21.71 ± 4.8</td>
<td>6.82</td>
<td>0.0307</td>
<td>0.102</td>
</tr>
<tr>
<td>600</td>
<td>30.15 ± 8.0</td>
<td>9.47</td>
<td>0.0426</td>
<td>0.0710</td>
</tr>
<tr>
<td>1000</td>
<td>58.38 ± 14.9</td>
<td>58.38</td>
<td>0.0816</td>
<td>0.0816</td>
</tr>
</tbody>
</table>

Table 3.4: Example of data from Izzo et al. (2007c) and resultant temperature and temperature gradients predicted by the model.

Other model parameters are $\lambda = 1850$ nm, $d_{\text{fibre}} = 500$ µm, $\phi_{\text{core}} = 200$ µm, NA = 0.22. The pulse length, radiant exposure, calculated peak temperature change and temperature gradient are shown in Table 3.4. The data points in Fig. 3.15 show the calculated peak temperatures and temperature gradients corresponding to the stimulation threshold results for CAP recordings in gerbil cochleae from Izzo et al. (2007c) and Richter et al. (2008). It appears that the thresholds for chronically deafened animals in Figure 3 of (Richter et al., 2008) have been incorrectly transcribed from Table 1 of the same reference. A previous publication (Thompson et al., 2013b) used the incorrect values, this has been corrected here. These calculated values use the cochlear model together with the geometry and laser parameters provided in the relevant references. When plotted in this way, the Izzo et al. (2007c) results visually suggest two regimes: for pulse length $\geq 100$ µs stimulation requires a minimum temperature gradient (i.e. energy over time or laser power) to achieve stimulation and below $\approx 100$ µs, where they suggest a minimum temperature (i.e. minimum pulse energy) is required. The Richter et al. (2008) control and acutely deafened result (Fig. 3.15 a,b) suggest a similar results, however the error bars are significantly larger than that observed by Izzo et al. (2007c).

The chronically deafened results from Richter et al. (2008) require an increasing laser power with longer pulse lengths. Additionally, the data has large error bars, making a meaningful comparison of the results difficult. The large variation between different animals may be due to the significant reduction ($\sim 60\%$) in spiral ganglion neurons due to the deafening process (Richter et al., 2008), thus requiring the exposure of a larger volume of tissue to achieve the same level of neural stimulation. Alternatively, greater positioning accuracy may be required when attempting to target the neurons.

A further comparison is made in Figure 3.16 with data from Izzo et al. (2008b), where a more strongly absorbed wavelength of 1937 nm was used to stimulate auditory neurons in a gerbil. Here the model used a distance of 55 µm between the fibre
Figure 3.15: a) Simulated peak temperature changes and b) average temperature gradient resulting from INS at a wavelength of 1850 nm, for pulse lengths between 10 µs and 2000 µs. Experimental stimulation thresholds from Izzo et al. (2007c) and Richter et al. (2008) are included for comparison.

Figure 3.16: a) Simulated temperature changes and b) Temperature gradient resulting from INS at a wavelength of 1937 nm, for pulse lengths between 10 µs and 1000 µs. Data from Izzo et al. (2008b) is included for comparison.
3.5. Microsecond Duration Pulses

Figure 3.17: Illustration of the four fits analysed for statistical significance.

and nerve similar to the distance reported by Izzo et al. (2008b). Note that these results are more sensitive to the model parameters, especially the fibre distance and absorption, due to the higher absorption level at this wavelength. This accounts for the larger temperature increases and gradient. The trend of two regimes on either side of 100 µs remains suggestive, as seen in Fig 3.15, although firm conclusions cannot be drawn as there is only one point above 100 µs.

Statistical analysis of the two regimes was undertaken using weighted least squares fitting against: two separate regimes; constant laser power; constant pulse energy; and a linear $y = ax + b$ line. A schematic illustration of these fits is shown in Figure 3.17. The error between the model and experimental results was weighted using the experimental standard deviation reported.

While the two regime fit had a lower sum of residuals than both a constant laser power or constant laser energy in all cases except the Richter et al. (2008) chronic data, the linear fit provided the lowest sum of residuals in all cases except the Izzo et al. (2007c) data. This suggests, while the two regime hypothesis is visually suggestive, it may not have a physical basis.

While a statistical analysis of these results do not support there being two distinct regimes of INS, there may still be multiple criteria underlying the response of INS. In the Izzo et al. (2007c) results, below approximately 100 µs, a minimum energy and therefore minimum temperature change is needed to evoke a response. Above approximately 100 µs and below approximately 1 ms, a minimum laser power and hence temperature gradient is required to drive a response. Existing published data does not provide enough data of sufficient accuracy to make statistically sound reliable conclusions about the existence of multiple regimes. It is also possible that there are multiple criteria for stimulation and that the response does not scale linearly with pulse length, as multiple biophysical processes may underly INS (Section 1.4.8). The data presented here also does not allow speculation about thresholds and mechanisms
for pulses of 1 ms or longer. For pulse lengths in this range (> 1 ms) there is a substantial decrease in the temperature gradient from a continuous power laser pulse, which may affect the amplitude of a capacitive change (Shapiro et al., 2012).

For a time duration of 100 µs, the thermal penetration depth $\delta_z$ is 7.5 µm, which is close to the size of a spiral ganglion neuron. Given steady state heating of tissue can result in thermally inhomogeneous areas (Chen and Wood, 2009), when the thermal penetration depth is on the same scale as the neurons affected, a different response may be observed when temperature is not uniform across the cell. For pulses of this duration, the heating could be as described adiabatic, as any temperature inhomogeneity over the cell may not even out.

This observation of both a temperature change and rate of temperature increase has also recently been made by Norton et al. (2013), as previously discussed in Section 1.4.9. They report a minimum temperature change of 0.8 m°C, temperature gradient of 0.0151 °C.ms$^{-1}$ and optimal pulse length of 53 µs to trigger neuronal activity. While the optimal pulse length is similar to that suggested here ($\sim$ 100 µs), the temperature change and rate of temperature change are much lower than suggested by this model. Here, the temperature increase 500 µm from the optical fibre is modelled, meaning that neurons further from the fibre which are involved with the response may be exposed to a lower temperature. Alternatively, the approach by Norton et al. (2013) aims to calculate the threshold for individual neurons by analysing the growth and saturation of the response.

Determining how these minimum temperature and temperature gradient requirements interact will require further experimental research to find the exact criteria for stimulation. Although the approach by Norton et al. (2013) and that presented here differ on the temperature calculated, they provide complementary approaches to understanding the detailed behaviour of heat during INS. Importantly, detailed characterisation of this energy dependence will allow for the minimum energy to be delivered to tissue and reduce laser peak power requirements, thus making development of any INS-based implants easier.

### 3.6 Conclusion

While the spatial distribution of light is important, the temporal behaviour is key to developing a full model of INS, especially due to the dependence of the process on the temporal rate of change in temperature. The finite element solution to the heat equation presented here provides key details of the behaviour of heat deposited during INS and allows for better understanding of longer pulses (> 1 ms) and the
3.6. Conclusion

thermal relaxation after pulses. Importantly it shows that minimising the area heated greatly reduces the thermal relaxation time and will be key in developing neural prostheses using INS. Additionally, the results from microsecond duration pulses in the cochlea suggest that there may be two regimes of INS. Further investigation of the effect could reduce the total optical energy required for neural activation and minimise the maximum laser power that must be delivered for INS.
Modelling of INS with multiple pulses

The temperature achieved by a single pulse and the resultant thermal behaviour, as discussed in Chapter 3, is important to understand, especially when the mechanisms of INS are being investigated. As many potential bionic applications of INS will use pulse repetitions (e.g. the cochlear implant (Clark, 2003)), a more complete picture of thermal behaviour during INS requires the model to take into account pulse repetitions and longer simulation durations. Current cochlear implants use electrical stimulation at a rate of up to 900 Hz (Clark, 2003). Although some experimental coding schemes allow for a lower overall rate of stimulation with full information delivery (Smith, 2013) and further research into coding schemes suggest that simulation rates in the order of 100 Hz may be feasible while maintaining high frequency fine structure (Dougherty and Richter, 2013), the thermal effects of stimulation rates up to 1 kHz must be considered by the model. Additionally, it is uncommon to encounter an isolated electrode in a bionic device, so to truly understand the effect of pulse repetition rates multiple emitters must be considered.

This chapter details the modification of the thermal model presented in Chapter 3 to allow for pulse repetitions, multiple emitters and modulations schemes. Section 4.1 presents the changes made to the model. Results of the model are investigated in Section 4.2, where impact of core diameter, pulse rate, multiple emitters and modulation schemes are studied. Section 4.3 discusses the influence of different
convective forms of heat transfer, which may change the resultant temperature over the longer time periods studied.

Portions of this chapter have previously been published in the following publication (Thompson et al., 2013a).

4.1 Modelling pulse repetitions

To evaluate pulse repetitions, longer time periods are required to be simulated. This requires changes to the model as described in Section 4.1.1.

4.1.1 Changes to the model to allow pulse repetitions

The model presented in this Chapter is an extension of the one presented in Chapter 3, with a number of extensions to allow for simulation of multiple pulses and multiple emitters.

To allow for multiple emitters, the total simulation size had to be increased. Rather than increasing the size of the matrix, therefore increasing the computational cost, the voxel size was increased to 10 μm, rather than 5 μm used in the previous chapter. With the same 256³ sized grid, the total simulation volume was increased to 2 × 2 × 2 mm. Additionally, increasing the voxel size increased the maximum stable timestep. When using a thermal diffusivity of $1.43 \times 10^{-7} \text{ m}^2\text{s}^{-1}$ (water), the maximum timestep now becomes $\Delta t_{\text{max}} = \frac{\alpha^2}{6\alpha} = 116 \mu s$. By using an increased simulation timestep compared to Chapter 3, longer total simulated times could be achieved without greatly increasing the running time. Longer simulated times are important as the temperature may continue to build up over a period of a few seconds, rather than the time periods < 100 ms considered in Chapter 3.

The model was modified to allow for multiple independent emitters with variable separation, as shown in Figure 4.1. Emitter separation was varied from 750 μm (analogous to the electrode spacing in current cochlear implants) to 250 μm, close to the point where 200 μm core diameter fibres would be touching. $d_{\text{fibre}}$ was kept constant at 500 μm. Only the guinea pig geometry is considered in detail, but the effect of different stimulation targets and geometries is considered in Section 4.3.

Typical simulations from Chapter 3 saved 100 2D slices at even time points over the whole time simulated. This was adequate for a 100 ms simulation, giving a 1 ms time step in the data analysed. However, given the longer time scales investigated here (≈ 10 s) saving only 100 slices would result in significant aliasing when modelling

\[ \text{With a timestep of 100 } \mu s, \text{ a 10 s simulation took } \sim 85 \text{ minutes, rather than } \sim 14 \text{ hours if a 10 } \mu s \text{ timestep was used.} \]
4.2 Influence of pulse repetition rate

Current cochlear implants use an electrical stimulation rate of up to 900 Hz (Clark, 2003). Due to modulation, the average rate may be lower, as the full rate is only used in bursts and depends on the frequency spectrum of the audio input (Clark, 2003). Typically, in current electrical implants, stimulation rates near 900 Hz are desired, as some patients report unwanted pitch perceptions such as buzzing when using stimulation rates in the range of 100 – 250 Hz (Hoesel and Clark, 1997). Recent research into stimulation rates (Smith, 2013; Dougherty and Richter, 2013) suggests that lower rates may be feasible without negative perceptions, but it remains unclear what the minimum stimulation rate may be.

Studies of INS in the cochlea have used stimulation rates of between 2 Hz and 250 Hz (Richter et al., 2011a; Rajguru et al., 2010; Izzo et al., 2007c). Izzo et al. (2007c) used repetition rates of up to 13 Hz in a gerbil cochlea and found no change in...
Figure 4.2: Flow diagram showing the operation of the FEM simulation after modification to allow for pulse repetitions.
in CAP response after 6 hours of continual exposure. Similarly, Rajguru et al. (2010) excited a cat cochlea for up to 10 hours at 200 Hz without any reduction in CAP amplitude or any damage to the spiral ganglion neurons in histology samples. In addition, a recent mini-review of the literature by Goyal et al. (2012), shows that pulse repetitions of up to 250 Hz can be used without any reduction in response amplitude or evidence of histological damage over periods of 6 hours.

Stimulation rates of up to 1000 Hz have been considered for a single emitter to represent the maximum frequency rate that may be needed in an implant. A maximum of 250 Hz was used to analyse the effect of multiple emitters and modulation schemes as higher rates were found to show a linear increase.

It is worth noting that INS in the cochlea requires a lower radiant exposure to generate a response compared to INS in peripheral models (Richter et al., 2011a). As such, the temperatures predicted by the model here are significantly lower than would be expected for peripheral stimulation at similar rates, but the trends of increasing stimulation rates is expected to apply to other stimulation modalities.

4.2.1 Single Emitter

Figure 4.3 shows an example of the heating in the spiral ganglion neurons modelled with a 200 μm core fibre located 500 μm from the neurons, pulse energy \( E_{\text{pulse}} = 25 \, \mu J \), pulse length \( t_p = 100 \, \mu s \) with repetition rates of 10 Hz, 50 Hz and 100 Hz. These laser parameters have been shown to generate action potentials in a number of studies (Goyal et al., 2012). A duration of 10 seconds was used as the peak temperature was found to have reached a plateau and stabilised. For comparison the heating from a single pulse is 0.11°C. After 10 seconds, the 10 Hz, 50 Hz and 100 Hz pulse rates have maximum temperatures of 0.17°C, 0.51°C and 0.95°C respectively. The increase in baseline temperature is similar to that observed by Wells et al. (2007a), where a two-fold increase in the peak temperature was observed when stimulating an exposed nerve at 5 Hz from a 600 μm core diameter fibre (Wells et al., 2007a), despite the different geometry and fibre diameter modelled here.

For the convenience of presentation and to allow for differences between pulse energies and other parameters the peak temperature after 10 s can be normalised against the increase from a single pulse. The resulting normalised peak temperature describes the increase in heating due to stimulation at a higher frequency.

Figure 4.4 shows the normalised peak temperature after 10 seconds of stimulation for fibre core diameters of 100 μm, 200 μm and 400 μm with the NA selected to keep the transition point \( z_T \) constant at 600 μm. Stimulation pulse rates of 1, 2.5, 5, 10, 25, 50, 100, 250 and 1000 Hz were selected to show the change between lower
Figure 4.3: Temperature at nerve 500 µm from fibre ($\varnothing_{\text{core}} = 200$ µm, NA = 0.22, $E_{\text{pulse}} = 25$ µJ with pulse rates of 10 Hz, 50 Hz and 100 Hz). Temperature increase from a single pulse is 0.11°C; peak temperature after 10 s is 0.95°C for stimulation at 100 Hz. a) shows the increase over the first second, while b) shows 10 seconds.

stimulation rates used in some studies (eg 13 Hz (Richter et al., 2011b; Izzo et al., 2007c)) to higher rates (eg 250 Hz (Rajguru et al., 2010; Goyal et al., 2012)).

Figure 4.4 shows that as the frequency of stimulation increases, so does the peak temperature and that smaller core diameter fibres show less of an increase in temperature when normalised to the increase from a single pulse. Above 10 Hz the 400 µm fibre shows a linear increase in temperature with frequency. When a smaller core diameter is simulated, this apparent linear trend does not occur until a higher stimulation frequency is used. The 100 µm fibre does not display a linear increase with temperature until approximately 100 Hz.

For $\varnothing_{\text{core}} = 200$ µm with a simulation rate of 250 Hz, pulse length $t_{\text{pulse}} = 100$ µs, wavelength of $\lambda = 1850$ nm and a pulse energy of $E_{\text{pulse}} = 25$ µJ, the model predicts a peak temperature of 2.3°C (or 20.7 times the peak of an individual pulse). These parameters are similar to those used by Goyal et al. (2012) which were not found to cause damage. While the wavelength used is different (Goyal et al. (2012) used 1869 nm), the minimal difference in temperature decay from single pulses of 1850 nm and 1870 nm shown in Chapter 3 suggests that the relative peak temperature achieved using 1869 nm will be very close to that of 1850 nm, although the absolute peak temperature will be different to that calculated here.

A fibre with 400 µm core diameter shows an increase of 80.5, while a 100 µm core diameter fibre only shows an increase of 8.8. This result can also be expressed in terms of frequency. For example, if it is required to restrict the peak temperature
4.2. Influence of pulse repetition rate

Figure 4.4: Normalised peak temperature after 10 s of stimulation (ratio of peak after 10 s compared to the peak temperature from a single pulse) for core diameters of 100 µm, 200 µm and 400 µm. The output is shown by the markers with a smoothing spline included as a guide to the eye.

4.2.1 Normalisation of the effect of core diameter

To 2.3°C, a 400 µm core diameter fibre achieves that temperature increase at just 63 Hz, while a 100 µm core diameter fibre has the same increase at 610 Hz.

From these results it is clear that localising light, through use of smaller fibre core diameters, greatly aids thermal relaxation and allows higher pulse repetition rates to be used.

The different between wavelengths of 1850 nm and 1870 nm is further examined in Figure 4.5. For a core diameter of 200 µm, only a minimal difference between the two wavelengths is observed, going from an normalised temperature of 20.7 times at 1850 nm to 21.5 at 1870 nm. There is a greater increase in the absolute temperature, 1850 nm has an increase of 2.3°C while the more strongly absorbed 1870 nm shows an increase 3.0°C. While the most strongly absorbed wavelength (1870 nm) may be expected to relax faster when using simple analytical model (e.g. (Izzo et al., 2008b)). The analysis of relaxation times from a single pulse in Chapter 3 shows that restricting the x and y axes of exposure have a greater impact on decay times than varying the wavelength used.

4.2.2 Multiple Emitter Spacing

Current cochlear implants use electrodes spaced at a pitch of at least 0.75 mm (Clark, 2003), although current spread in tissue may make the effective separation greater. To investigate the effects of multiple stimulation sites, the model was modified to allow for simultaneous stimulation at three sites, with pitch spacing between 0.25 mm and 0.75 mm. The following parameters were used: \( \lambda = 1850 \text{ nm}, \ \varnothing_{\text{core}} = 200 \text{ µm}, \)
Figure 4.5: Normalised peak temperature after 10 s of stimulation (ratio of peak after 10 s compared to the peak temperature from a single pulse) for wavelengths of 1850 nm and 1870 nm (\( \varnothing \text{core} = 200 \, \mu\text{m}, \text{NA} = 0.22 \)).

NA = 0.22, pulse energy = 25 \( \mu \text{J} \) and pulse length = 100 \( \mu \text{s} \).

Figure 4.6 shows the change in temperature from stimulation with a single emitter at 100 Hz and three emitters separated by distances of 750 \( \mu \text{m} \), 500 \( \mu \text{m} \) and 250 \( \mu \text{m} \). The reported temperature for the multiple emitters was taken from the central emitter. After 10 seconds of stimulation, the single emitter has a peak temperature of 0.95\( ^\circ \text{C} \), while the peak temperature for the multiple emitters was 1.15\( ^\circ \text{C} \), 1.35\( ^\circ \text{C} \) and 1.80\( ^\circ \text{C} \) for separations of 750 \( \mu \text{m} \), 500 \( \mu \text{m} \) and 250 \( \mu \text{m} \) respectively. Reduced separation results in a correspondingly greater increase in temperature.

Again, a greater range of frequencies can be analysed by comparing the normalised peak temperature. Figure 4.7 shows the peak temperature after 10 seconds, normalised against the peak temperature achieved by a single pulse in a single fibre and when three fibres are positioned 750 \( \mu \text{m} \), 500 \( \mu \text{m} \) and 250 \( \mu \text{m} \) apart. When multiple stimulation sites are positioned closer together, the peak temperature rises. At 250 Hz, with a single stimulation site, the peak temperature after 10 seconds is 20.7 times a single pulse’s peak temperature. When three emitters are used the ratio of peak temperatures becomes 24.9, 29.3 and 39.3 for separation distances of 750 \( \mu \text{m} \), 500 \( \mu \text{m} \) and 250 \( \mu \text{m} \) respectively. Frequencies higher than 250 Hz are not shown as peak temperatures continue to increase linearly with frequency.

Similar to the results for different fibre diameters, the temperature increase for multiple emitters can also be considered in terms of the frequency that limits the temperature increase to a specified level. The frequencies which display the same temperature increase of 20.7 relative to a single pulse are 207 Hz, 175 Hz and 130 Hz for the respective separation distances of 750 \( \mu \text{m} \), 500 \( \mu \text{m} \) and 250 \( \mu \text{m} \).
4.2. Influence of pulse repetition rate

Figure 4.6: Temperature in the nerve layer 500 µm from the fibre ($\phi_{core} = 200$ µm, NA = 0.22, $E_{pulse} = 25$ µJ at a pulse rate of 100 Hz) with a single emitter and three stimulation sites with a separation of 750 µm, 500 µm and 250 µm. Temperature increase from a single pulse is 0.11°C; peak temperature after 10 s is 0.95°C for a single emitter and 1.80°C for three emitters separated by 250 µm. a) Shows the increase over the first second, while b) shows 10 seconds.

Figure 4.7: Normalised peak temperature after 10 s of stimulation (ratio of peak after 10 s compared to the peak temperature from a single pulse). Three stimulation sites with a separation of 750 µm, 500 µm and 250 µm are included. The peak temperature was taken from the central emitter.
Figure 4.8: Dependence of normalised peak temperature (ratio of peak after 10 s compared to the peak temperature from a single pulse) on varying separation between three emitters with stimulation rates of 50 Hz, 75 Hz, 100 Hz and 125 Hz. A wavelength of $\lambda = 1850$ nm and fibre core diameter of 200 $\mu$m was used. The temperature was taken from the central emitter.

The increase in temperature due to emitter spacing can be seen more clearly by plotting the temperature ratio against the emitter spacing. Figure 4.8 shows the ratio of peak temperature after 10 seconds of stimulation to that of a single pulse when using a fibre with a core diameter of 200 $\mu$m, $\lambda = 1850$ nm, $E_{\text{pulse}} = 25$ $\mu$J and pulse rates of 50 Hz, 75 Hz, 100 Hz and 125 Hz. These frequencies were chosen to allow visualisation on a linear scale. The temperature increase when emitters are separated by 500 $\mu$m with a stimulation rate of 100 Hz, is approximately equivalent to a stimulation rate of 75 Hz with emitter separation of 250 $\mu$m or stimulation rate of 120 Hz with emitter separation of 800 $\mu$m.

Additionally, when using a separation distance of 250 $\mu$m there is significantly more heat build up between the emitters, which may cause stimulation in regions not directly targeted by INS. Figure 4.9a) shows the temperature at the target nerves and at sites 750 $\mu$m, 500 $\mu$m and 250 $\mu$m laterally displaced from the centre of the beam for a single emitter. The temperatures outside the directly exposed region do not display a rapid increase in temperature. As INS is thought to be dependent on a temperature gradient in time (Shapiro et al., 2012), this indicates that stimulation is likely to be localised in the region directly exposed to the laser radiation. Sites further from the stimulation show reduced temperature increases. After 10 seconds, the targeted nerves reach a peak temperature of 0.14°C, while non-stimulated sites 250 $\mu$m, 500 $\mu$m and 750 $\mu$m away increase by only 0.023°C, 0.010°C and 0.005°C respectively. As Figure 4.9b) shows, when multiple emitters are used the temperature increase is greater. Here multiple emitters are used with a spacing of 750 $\mu$m.
4.2. Influence of pulse repetition rate

Figure 4.9: Temperature in the nerve layer 500 µm from fibre (core diameter = 200 µm, NA = 0.22, \( E_{\text{pulse}} = 25 \) µJ with a pulse rate of 5 Hz). (a) Also shown are the temperatures at sites without stimulation 750 µm, 500 µm and 250 µm away from the optical axis. Temperature increase from a single pulse = 0.11°C, peak temperature after 10 s = 0.137°C. (b) Same as a) but with three emitters with a separation of 750 µm. Temperature increase from a single pulse = 0.11°C, peak temperature after 10 s = 0.147°C.

The temperature is taken from the central emitter at non-stimulated sites 250 µm and 500 µm away and stimulated site 750 µm away, giving a temperature rise of 0.035°C, 0.033°C and 0.14°C respectively.

Modulation Schemes

Any cochlear implant or other application of INS is unlikely to use all emitters at their maximum rate, therefore it is worth investigating the effect of different modulation schemes on the final temperature increase. Figure 4.10 shows three different modulation schemes that were investigated together with a 100% duty cycle pulse stimulation pattern for comparison. In addition to the 100% duty cycle, three different modulations schemes are used: one where all three emitters fire simultaneously but at a rate of one third that of the full rate, giving a 33% duty cycle; one where each emitter fires in turn, giving an average rate duty cycle of 33% (1/3 Modulation); and a burst rate, where each emitter in turn fires 10 times at the stimulation frequency, again giving an overall duty cycle of 33% (10/30 Modulation).

An example of the simulated heating from the modulation schemes at 100 Hz is shown in Figure 4.11. The 33% scheme is not shown as it appears the same as 1/3 modulation. Even though the 1/3 and 10/30 schemes have the same number of pulses in 1 s, the 10/30 scheme has a much higher peak temperature of 0.65°C compared...
Figure 4.10: Diagram showing the 4 modulation schemes examined, a filled red circle indicates that the laser is “on” for that emitter, while a small unfilled circle indicates that the laser is “off”. 100% modulation has all emitters on simultaneously; 33% has all emitters on simultaneously but at 1/3 of the rate; 1/3 modulation has each emitter on individually in sequence; 10/30 modulation has each emitter in turn giving a burst of 10 pulses for an overall rate of 33%.
4.3 Convective cooling

So far, only heat loss through conduction has been considered. Previously, only heat conduction was considered due to the low interaction time ($t < 1$ s) investigated.

Figure 4.11: Temperature at nerve 500 $\mu$m from fibre ($\phi_{\text{core}} = 200$ $\mu$m, $\text{NA} = 0.22$, $E_{\text{pulse}} = 25$ $\mu$J) at a pulse rate of 100 Hz with three emitters separated by 750 $\mu$m and three different modulation schemes. 100% modulation has a peak temperature of 1.15°C, 1/3 modulation 0.43°C and 10/30 modulation 0.65°C. a) shows the increase over the first second, while b) shows 10 seconds.

to 0.43°C for 1/3 modulation. The increase in temperature for 10/30 is due to the rapid pulses in quick succession. 100% is higher again at 1.15°C, an increase of 2.7 times the 1/3 modulation. This increase is the same as would be expected going from 100% modulation at 33 Hz to 100 Hz based on the results shown in Figure 4.7.

Figure 4.12 shows three different modulation cases compared to a 100% duty cycle pulse stimulation pattern using the normalised peak temperature. Again, the parameters are as used previously ($E_{\text{pulse}} = 25$ $\mu$J, $\lambda = 1850$ nm, $t_p = 100$ $\mu$s). From the results in Figure 4.12, there is no difference between the modulation cases where the emitters fire simultaneously or once each; for these cases the overall temperature change is the same as a stimulation rate of one third. However, the burst stimulation (10/30 modulation) shows a larger temperature increase. However, based on the results shown in Figure 4.11 this is only an increase in the peak temperature and the average temperature is similar to 33% and 1/3 modulation (0.339°C vs 0.336°C). This shows that the thermal load of both long term average and burst stimulation rates need to be considered in the design of any INS based implant.

4.3 Convective cooling

So far, only heat loss through conduction has been considered. Previously, only heat conduction was considered due to the low interaction time ($t < 1$ s) investigated.
Figure 4.12: Normalised peak temperature (ratio of peak after 10 s compared to the peak temperature from a single pulse) for different modulation schemes, assuming three emitters separated by 750 µm.

This is a reasonable assumption for typical laser-tissue interactions due to the shorter interaction times and low temperatures generated (Niemz, 2007). Due to the low temperatures, it is still unlikely that radiation will be significant form of heat transfer, as such remains discounted. However, when examining the temperature increase during pulse repetitions, it is likely that the total interaction time will be much longer, up to the order of hours in cases like the cochlear implant. Given INS has been proposed for use in implanted devices (Richter et al., 2011a), where interaction times are measured in terms of minutes, hours or days, it is reasonable to also consider the contribution that convection may play.

Four broad forms of convection are considered in this section: natural-forced convection, where blood or other fluids flow through the target tissue; forced convection, where fluid is run over the target tissue during or after stimulation, such as when the tissue is exposed; unforced convection where convection occurs due to temperature differences driving natural movement of the liquid or air; and convective losses through evaporation of water. In the context of INS, these forms of convection manifest themselves in a variety of targets and processes. Some of these processes are general to all targets (e.g. the perfusion of blood or other fluids in the target tissue). In the cochlea, the natural flow of perilymph through the scala tympani and the formation of convection cells in the scala tympani perilymph need to be considered. For exposed nerve experiments, such as sciatic (Wells et al., 2007a) or brain (Cayce et al., 2011), heat driven evaporation of water and convection of air near the tissue could influence the outcome.

This section considers the contribution to heat flow and cooling of these different
4.3. Convective cooling

Tissue Perfusion Rate ($\text{mL.min}^{-1}\cdot\text{g}^{-1}$)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Perfusion Rate ($\text{mL.min}^{-1}\cdot\text{g}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>0.012 – 0.015</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.07 – 0.2</td>
</tr>
<tr>
<td>Skin</td>
<td>0.15 – 0.5</td>
</tr>
<tr>
<td>Brain</td>
<td>0.46 – 1.0</td>
</tr>
</tbody>
</table>

Table 4.1: Perfusion rates for different tissues. Data from (Niemz, 2007).

convection mechanisms. The calculated loss terms are not intended to be highly accurate, but rather to provide an estimate of the loss rates to assess the relative importance the magnitude of the loss compares to the heat introduced by the laser and removed by thermal conduction.

4.3.1 Perfusion of liquid in tissue

Perfusion of blood or other fluid inside in tissue could cause a form of forced convection. In the the cochlea, this movement of fluid could potentially cool the spiral ganglion neurons or other INS targets, by transferring heat away from the target to the rest of the body. While blood flow in the cochlea has been studied in some detail (Mom et al., 2005), only relative flow is typically measured (Mom et al., 1999). Lacking perfusion rates for neural tissue in the cochlea, typical values for general tissue (Shown in Table 4.1) are used instead as a first approximation. Niemz (2007) provides a range of 0.46 - 1.0 $\text{mL.min}^{-1}\cdot\text{g}^{-1}$ for the brain.

The quantity of heat energy removed by perfusion in tissue can be estimated by taking the rate of perfusion for the tissue ($F_v$), the heated or irradiated volume ($V_i = 1.25 \times 10^{-4}$ mL), temperature increase in the tissue ($\Delta T$), heat capacity of the tissue ($C_p = 4.18$ J.g$^{-1}.\text{°C}^{-1}$) and density of water ($\rho_w = 1$ g.mL$^{-1}$) and tissue ($\rho_t = 1$ g.mL$^{-1}$). The rate of heat loss is given by:

$$E = \Delta T C_p V_i \rho_w F_v \rho_t.$$  \hspace{1cm} (4.1)

For a temperature rise of $\Delta T = 3$ °C, approximately corresponding to 25 $\mu$J pulses at a 250 Hz repetition rate, and a perfusion rate of $F_v = 1.0$ $\text{mL.min}^{-1}.\text{g}^{-1}$, this gives a convective heat transfer rate of 1.58 mJ.min$^{-1}$. This potential convective energy flow is small in comparison to the energy introduced by the laser and assumed to be conducted out of the irradiated region at equilibrium, $E = 60 f E_{\text{pulse}} = 375$ mJ.min$^{-1}$.

Therefore, the perfusion of blood through the cochlea or other tissues is unlikely to provide a significant cooling effect for high repetition rate stimulation. As values for perfusion rates in other tissues are generally lower than that of the brain (Table 4.1), it is reasonable to assume the contribution to heat transfer from blood perfusion
in other target structures, such as the sciatic nerve, is similar to or less than that considered here.

### Cochlear Fluid Flow

Compared to most tissue, the cochlea has chambers full of fluid, which could increase the potential for convective cooling. These chambers (scala tympani, scala vestibuli and scala media), naturally have fluid flowing through them. When the cochlea is damaged, such as when a cochleostomy is performed during an INS experiment, this flow rate increases. Any additional source of liquid flow could increase the loss of heat through convection.

When the cochlea is undamaged, the volume flow of fluid (i.e. perilymph) in the scala tympani is very low (Mynatt et al., 2006); experimental measurements report flows in the range of $F_p = 1.6 \text{nL.min}^{-1}$ (Ohyama et al., 1988). In INS experiments where light is directed to the spiral ganglion neurons through the undamaged round window (e.g. Izzo et al. (2007c)), this lower value is a reasonable approximation of perilymph flow rates. The other primary approach to the spiral ganglion neurons is through a cochleostomy (e.g. Goyal et al. (2012)), this damages the cochlea and may increase perilymph flow. In a damaged cochlea, perilymph flow can rise to $F_p = 0.5 \mu\text{L.min}^{-1}$ (Salt et al., 1991).

The convective heat transfer can be estimated by assuming that all of the perilymph flow is taking heated fluid away, this represents a greatest flow rate scenario. Energy lost can be calculated by:

\[ E = \Delta T F_p C_p \rho \]  

For a temperature rise of $\Delta T = 3^\circ\text{C}$ and flow rate for an undamaged cochlea ($F_p = 1.6 \text{nL.min}^{-1}$), this gives an energy removal rate of $E = 20 \mu\text{J.min}^{-1}$. This flow rate is significantly lower than that estimated for blood and general tissue perfusion and is therefore too low to provide significant cooling and will have less influence on the temperature change than blood flow. In a damaged cochlea, the perilymph flow can be significantly higher, so the convective cooling from this process may be greater than that due to blood flow. For a flow rate of $0.5 \mu\text{L.min}^{-1}$, the perilymph could allow heat transfer of $E = 6.3 \text{mJ.min}^{-1}$, which is still significantly lower than the loss due to conduction. Therefore, it is unlikely that the temperatures achieved in the experiments discussed by Goyal et al. (2012) are greatly reduced due to perilymph flow out of the cochleostomy. However, care must be taken when extrapolating the differences due to perilymph flow, as the experimental arrangement may significantly
change this flow rate and the resultant temperatures.

### 4.3.2 Unforced convection in the cochlea

In the cochlea, it is possible that heating from the laser could generate convection cells inside the scala tympani perilymph and could also assist in cooling. Convection cells are strongly dependent on geometry and orientation, as they are driven by differences in buoyancy (Bergman and Incropera, 2011). Therefore, it is difficult to model a realistic case as the geometry will vary between experimental subjects and neuronal targets. However, the feasibility of this process can be estimated by taking a case where convection is most likely to occur. The Rayleigh number \( Ra \) describes the primary form of heat transfer in a fluid. When the Rayleigh number is below the critical Rayleigh number for the fluid most heat transfer will be conductive, but when it is greater than the critical value, convection can play a role in heat transfer. The Rayleigh number is given by:

\[
Ra = \frac{g\beta}{\nu\alpha}(T_s - T_\infty)L^3
\]  

(4.3)

where the acceleration due to gravity \( g = 9.8 \text{ m.s}^{-1} \), \( \beta = 3.62 \times 10^{-4} \text{ °C}^{-1} \) is the thermal expansion coefficient of water (at 37 °C) (Bergman and Incropera, 2011), \( \nu = 6.61 \times 10^{-7} \text{ m}^2\text{s}^{-1} \) is the kinematic viscosity of water (at 37 °C) (Bergman and Incropera, 2011), \( \alpha = 1.43 \times 10^{-7} \text{ m}^2\text{s}^{-1} \) is the thermal diffusivity, \( T_s - T_\infty \approx 3 \text{ °C} \) is the temperature difference between baseline temperature and the maximum temperature due to the laser and \( L \) is the distance between the heated fluid and the boundary of the fluid and is between 200 \( \mu \text{m} \) and 500 \( \mu \text{m} \), depending on the location of the stimulation in the cochlear spiral. For these values, a Rayleigh number of \( Ra = 0.901 \) for \( L = 200 \mu \text{m} \) and \( Ra = 14.1 \) for \( L = 500 \mu \text{m} \) is found. The critical Rayleigh number for water depends on the temperature and geometry, however even a lower bound value of \( Ra_c = 1700 \) (Heitz and Westwater, 1971) is still much greater than the Rayleigh number found for the temperature and geometry relevant to INS in the cochlea. Therefore, thermally driven natural convection is unlikely to provide a significant cooling mechanism during INS of the cochlea.

### 4.3.3 Forced convection in the air

So far, only forms of convection which can occur when the body is a closed and not open to the environment have been considered. In experiments where the target tissue is open to the environment, it is possible that conditions in the room may influence
the resultant temperature. Although these cases are unlikely to be relevant for long
term use, such as in an implant, there may be a difference between exposed and
enclosed targets. This difference would be important in determining safe thresholds.

Experimental arrangements with exposed nerves present two sources of cooling
through forced convection: circulation of cooler room temperature air and the
addition of saline solution to prevent tissue dehydration (e.g. Duke et al. (2012a)).
These arrangements are typically required for peripheral nerves, which have higher
thresholds for stimulation and use lower stimulation rates.

If there is forced convection of air, any contribution is likely to be greater when
evaporation of water is considered. The calculation of evaporation in Section 4.3.4,
includes consideration of the air speed near the target and therefore includes the
contribution of forced convection.

For the saline solution case, unless the solution is continuously flowing over
the tissue it is unlikely to provide a major source of cooling to the tissue during
stimulation. However, cooling of the tissue to below body temperature may contribute
to a weaker response at the start of pulse trains as observed by Duke et al. (2012a).

4.3.4 Evaporation of water

In an exposed experiment, heat could also be lost through evaporation of water.
Evaporation involves both the convective flow of water vapour away from the tissue
and loss of heat through vaporisation.

To evaluate the effect of evaporation on the resulting temperatures, Stelling’s
formula for evaporation can be used (Welch and van Genert, 2011; Torres et al.,
1993) to find the heat loss through vaporisation. The vaporisation loss term can be
given as

\[ Q_{vap} = \zeta h_{fg}\rho_w \left[ Wm^{-2} \right], \]  \hspace{1cm} (4.4)

where \( h_{fg} \) is the phase change enthalpy for water, \( \rho_w \) is the density of water and \( \zeta \) is
the surface loss rate. The surface loss rate \( (\zeta) \) is given by Stelling’s formula:

\[ \zeta = (A_s + B_s U)[P_s(T) - P_s(T_e, RH)], \]  \hspace{1cm} (4.5)

where \( A_s = 7.31 \times 10^{-11} \text{ m Па}^{-1} \text{s}^{-1}, B_s = 1.2 \times 10^{-11} \text{ Па}^{-1}, U \) is the free stream air
velocity above the surface, \( P_s(T) \) is the saturation pressure at tissue temperature
and \( P_s(T_e, RH) \) is partial pressure of water vapour in air at temperature \( T_e \) with
relative humidity \( (RH) \).

To evaluate the potential loss of heat through evaporation, a case is taken where
evaporation is expected to be very high due to an increased temperature. Using the
above formula, if tissue in a 400 $\mu$m radius is heated to 60 °C with an air temperature 25 °C, RH = 25% and air velocity $U = 1$ m.s$^{-1}$, then an evaporative cooling rate of 461 $\mu$W or 27.7 mJ.min$^{-1}$ is found. With a smaller temperature increase of 5 °C ($T_s = 42$ °C), Eqs (4.4) and (4.5) predict an evaporative cooling rate of 179 $\mu$W or 10.7 mJ.min$^{-1}$. If an increased air velocity of 10 m.s$^{-1}$ is used, the loss increases by 41% to 252 $\mu$W or 15.1 mJ.min$^{-1}$. As a laser heating rate of 375 mJ.min$^{-1}$ gives a temperature increase of only 2.3 °C (at 500 $\mu$m depth), it is therefore reasonable to conclude that heat loss through evaporation makes a minimal contribution when tissue temperatures remain at safe levels, but it is possible that it plays a role in reducing the surface temperature when high radiant exposures are used.

### 4.3.5 Media interfaces

Forced convection has been considered in Sections 4.3.3 and 4.3.4, however the change in temperature from conduction has not been considered over longer time periods. Section 3.3.1 investigated the change in temperature when near a non-absorbing media. Increased proximity resulted in a lower peak temperature, when the medium selected was glass. Glass has a thermal diffusivity on the same order of magnitude as water ($\alpha_{\text{water}} = 1.43 \times 10^{-7}$ m$^2$.s$^{-1}$, $\alpha_{\text{glass}} = 3.7 \times 10^{-7}$ m$^2$.s$^{-1}$) so similar thermal behaviour is expected. However, air has a much lower thermal diffusivity ($\alpha_{\text{air}} = 2.0 \times 10^{-5}$ m$^2$.s$^{-1}$) and low thermal conduction, which means that it may partially insulate the tissue and reduce the potential for heat to flow away. This reduced conduction could potentially increase the heat build up in the tissue, resulting in a temperature increase greater than predicted.

![Figure 4.13: Tissue interfaces](image)

To test the model for INS in cases where an air-tissue interface is present (as shown in Figure 4.13), the model was modified to simulate an optical fibre positioned 100 $\mu$m away from the tissue, in air. To ensure that the difference reported by the model was due to thermal conduction and not a different beam profile, the
absorbed heat profile was kept the same as the one used in the water-tissue geometry. The thermal diffusivity of the glass fibre was set to $\alpha = 3.7 \times 10^{-7} \text{ m}^2\text{s}^{-1}$ and $\alpha = 2.0 \times 10^{-5} \text{ m}^2\text{s}^{-1}$ for air. The initial temperature of the air and fibre was assumed to have stabilised at the same temperature as the tissue.

Figure 4.14 shows the temperature increase from a 200 $\mu$m core diameter fibre at a stimulation at 50 Hz at a distance of 50 $\mu$m in the tissue layer over one second. The air-tissue geometry shows an increased temperature of 0.565 °C compared to 0.527 °C for the water-tissue geometry. Figure 4.15 shows the normalised peak temperature after 1 s of stimulation along the z-axis. 1 second was used rather than 10 seconds, as a full 10 second simulation would have taken a prohibitively long time\(^3\).

![Figure 4.14: Temperature change over 1 second, with the temperature taken 50 $\mu$m from the tissue interface.](image)

Using with stimulation rate of 50 Hz, no significant difference was observed between the air-tissue and water-tissue geometries at tissue depths greater than 250 $\mu$m. However, a slightly higher temperature was observed in the air-tissue geometry at reduced depths. At the smallest distance examined (50 $\mu$m), the normalised peak temperature increased by 7.2% from 3.04 to 3.26 after 1 s of stimulation.

The small difference in the predicted temperature change between the air-tissue and water-tissue model is unexpected. Air has a very low thermal conductivity (Welch and van Gemert, 2011) and acts as an insulator, reducing heat flow. This reduced thermal conductivity is expected to increase the temperature compared to the water-tissue case, where water will be more effective at conducting heat away. While the fixed boundary conditions were found to be adequate for modelling of heat flow in water

\(^3\)Thermal diffusivity of air ($\alpha = 2.0 \times 10^{-5} \text{ m}^2\text{s}^{-1}$), gives a maximum stable time step of 0.833 $\mu$s, resulting in a simulation time of $\sim$ 8 days.
4.4 Conclusion

The results presented in this chapter give predictions of the temperature that tissue is exposed to during INS at high repetition rates and shows the influence of controlling the spatial localisation, repetition rate and modulation scheme. For the fibre core diameters studied (100 µm – 400 µm) the resultant temperature is always less than what would be expected by simply summing the temperature increase from individual pulses together. Reducing the irradiated area, by adjusting the fibre core diameter, reduces the maximum temperature. When multiple emitters are used, the temperature increases further compared to a single emitter. The temperature increase is greater when emitter spacing is reduced. Different modulation schemes have an influence on the resulting temperature, which implies that any INS based implant will have to take into account both peak and average temperatures, depending on the encoding scheme used. Overall, convective heat transport processes were not found to provide a significant form of additional heat transport for the cases considered.
here.
Part II

Infrared Stimulation in the Cochlea
This chapter introduces the lasers used in experimental work to stimulate the cochlea with INS and the relevant experimental methods used.

A range of different laser sources have been used for INS, with varying wavelength, peak power levels and optical fibre types used for delivery to the region of interest. As previously discussed in Chapter 2, most lasers used for INS produce a wavelength corresponding to a water absorption coefficient in or near the range of $\mu_a = 1-2 \text{ mm}^{-1}$. This water absorption coefficient corresponds to lasers with wavelengths of 1850 – 1870 nm and 2120 nm, which have been used by Wells et al. (2005a) and Izzo et al. (2006, 2007c) amongst others. This wavelength range provides a compromise between the penetration of light into the tissue and the absorption required to trigger neural stimulation.

Since INS lasers require specific wavelengths and high minimum power densities, a number of laser sources were developed in this project to investigate INS in the cochlea. This chapter details the development of laser sources used for INS and the characterisation and experiments performed with them. Section 5.1 introduces the laser sources and the specific reasons for their use. Section 5.2 details the experimental methods used for the animal studies in Part II of this thesis.

To date, published results of INS in the cochlea have almost exclusively come from
the Richter group at Northwestern University\textsuperscript{1}. While the technique for performing INS appears straightforward, attempts to stimulate the cochlea with INS, as discussed in Chapters 5 and 6, have not always been successful despite having laser sources within the published thresholds.

## 5.1 Laser sources

A number of laser sources have been used in an attempt to demonstrate stimulation of the cochlea using INS in this thesis. A summary of the laser sources is presented in Table 5.1.

<table>
<thead>
<tr>
<th></th>
<th>MK I</th>
<th>MK II</th>
<th>MK III</th>
<th>MK IV</th>
<th>Aculight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>1450</td>
<td>1870 – 1900</td>
<td>1850 – 1870</td>
<td>1550</td>
<td>1869 – 1889</td>
</tr>
<tr>
<td>Core Diameter (µm)</td>
<td>SM\textsuperscript{2}</td>
<td>600</td>
<td>200</td>
<td>105 – 200</td>
<td>200</td>
</tr>
<tr>
<td>NA</td>
<td>0.13</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Power (mW)</td>
<td>150</td>
<td>380</td>
<td>360</td>
<td>3000</td>
<td>1520</td>
</tr>
<tr>
<td>Radiant Exp. (mJ.cm\textsuperscript{-2})</td>
<td>29,800\textsuperscript{3}</td>
<td>13.4</td>
<td>115</td>
<td>955</td>
<td>484</td>
</tr>
<tr>
<td>Absorption $\mu_a$ (mm\textsuperscript{-1})</td>
<td>2.6</td>
<td>1.9 – 8.1</td>
<td>0.95 – 1.9</td>
<td>0.96</td>
<td>1.9 – 5.5</td>
</tr>
<tr>
<td>$\Delta T$ @ 250 µm (°C)</td>
<td>1.90\textsuperscript{4}</td>
<td>0.036\textsuperscript{5}</td>
<td>0.37</td>
<td>1.75</td>
<td>1.41</td>
</tr>
<tr>
<td>$\Delta T$ @ 500 µm (°C)</td>
<td>0.286</td>
<td>0.005</td>
<td>0.20</td>
<td>1.37</td>
<td>0.88</td>
</tr>
<tr>
<td>Pulse Durations Used (ms)</td>
<td>0.1 – 1</td>
<td>0.1 – 2</td>
<td>0.1 – 2</td>
<td>-</td>
<td>0.1 - 10</td>
</tr>
<tr>
<td>Response Observed</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 5.1: Summary of light sources used for INS experiments in this work. The radiant exposure and theoretical temperature increases are for a 100 µs pulse at maximum power, at 250 µm or 500 µm.

The maximum radiant exposure generated by each laser across different pulse durations, compared to the experimental thresholds for different optical stimulation modalities is shown in Figure 5.1. Approximate threshold ranges for different optical stimulation modalities are highlighted with coloured ovals. At a given pulse length, each laser is able to generate a radiant exposure at or below the of its line. For a full description of the experimental thresholds see Figure 1.9. The radiant exposure for first laser developed (MK I) is much greater near the fibre tip due to the small

\textsuperscript{1}The only other published report of INS in the cochlea is a conference abstract as discussed in Section 1.4.3.

\textsuperscript{2}Single mode fibre with a mode field diameter $\sim$ 8 µm.

\textsuperscript{3}Radiant exposure is for a single mode fibre, assuming a mode field diameter of 8 µm, compared to multimode fibres, this will decrease much faster and is not comparable expect at the fibre tip.

\textsuperscript{4}Temperatures were calculated using an analytical model for laser MK I due to the single mode fibre. A uniform spread of light is assumed.

\textsuperscript{5}Temperature at 100% power. Maximum temperature change was at a lower power level due to a wavelength shift with increasing current causing absorption before the target to increase.
5.1. Laser sources

Figure 5.1: Comparison between the maximum radiant exposure of the lasers used in this study and a summary of threshold parameters reported for INS and other optical neural stimulation modalities (from Fig 1.9). Each laser is able to generate radiant exposure at or below/to the right of its line. Cross markers show the individual experimental thresholds from different papers, while the coloured ovals indicate the clustering of different modalities.

Figure 5.2: Comparison of the wavelengths and resultant absorption ranges for the five different lasers discussed in this chapter, showing a wider wavelength range (1400 – 2200 nm) and an expansion of the range 1840 – 1900 nm. Absorption for commonly used wavelengths of 1850, 1870 and 2120 nm are shown with a red star. Absorption of lasers without a wavelength range are shown with a green triangle (MK I and MK IV).
core diameter. Compared to the other lasers in multimode fibres, this will rapidly reduce as the beam expands. Although this comparison between the lasers does not take into account their wavelengths and resultant differences in water absorption coefficients, it allows for a simple evaluation of how the different lasers compare to published thresholds in different modalities.

A comparison of the absorption characteristics of the five lasers developed is shown in Figure 5.2. The figure also shows the commonly used INS wavelengths of 1850, 1870 and 2120 nm, along with the desired absorption range of 1 − 2 mm\(^{-1}\) (see Section 2.2.2) highlighted in blue, which provides a balance between absorption and penetration depth. As only a central wavelength for lasers MK I and MK IV was provided by the manufacturers, these are marked with a green triangle to show their water absorption. The other lasers (MK II, MK III and aculight) have their range illustrated with filled regions. Red with ‘\’ hatching shows laser MK II, Green with ‘/’ hatching shows laser MK III and and yellow with ‘|’ hatching shows the aculight laser. All of the lasers used are either partially or completely outside the suggested water absorption range of 1 − 2 mm\(^{-1}\), however all are close to the range and the commonly used wavelengths of 1850 and 2120 nm are also just outside the desired range.

Measurement of laser parameters was performed with the following devices. Pulse shape was measured with a Thorlabs DET10D biased photodiode connected to a Tektronix TDS 3054 digital oscilloscope, which was used for data acquisition. The photodiode was separated by an adequate distance from the laser emitter to prevent saturation at 100% power. Laser power was measured with a Coherent Fieldmaster and Coherent Model LM-10 detector head. Not all lasers could be run in continuous wave (CW) mode. For lasers that could only be pulsed, the duty cycle was measured along with the power and the equivalent CW power was calculated.

5.1.1 MK I: 1450 nm laser

The first laser developed for INS was a 1450 nm diode laser, coupled to a single mode fibre (QPhotonics, QFBGLD-1450-150). The wavelength of 1450 nm was selected to be closer to the telecommunication wavelength range, as a larger number of fibres, fibre tools and light sources exist in this wavelength range compared to the 1850 nm range. Although this wavelength provided increased water absorption compared to 1850 nm (2.6 mm\(^{-1}\) vs 0.95 mm\(^{-1}\)) it is still close to the range used for previous demonstration of INS in the cochlea and other targets. A water absorption coefficient of \(\mu_a = 2.6 \text{ mm}^{-1}\) corresponds to a wavelength of \(\lambda = 1877 \text{ nm}\) or \(\lambda = 2102 \text{ nm}\), in the range of previously used lasers.
5.1. Laser Sources

The diode was driven by a Newport Model 560 Laser Diode Driver and was temperature controlled by a Newport Model 350 Temperature Controller. The current delivered to the diode, and therefore power emitted, was controlled by a signal input to the Newport Model 560 Laser Diode Driver, with an external modulation transfer function of 300 mA.V⁻¹ or 600 mA.V⁻¹. The pulse length and frequency was controlled by an external signal generator.

Although the power of this laser was significantly lower than the maximum used for INS in the published literature (150 mW vs 1.4 W)\(^6\), near the fibre tip, it has an increased radiant exposure compared to other INS lasers due to the small optical fibre core diameter. It was postulated that the increased water absorption (2.6 mm⁻¹) and increased radiant exposure at short distances would compensate for the reduced power.

Calculating the radiant exposure from a single mode fibre is more complex than the multimode fibre case. Unlike the multimode fibre, the radiant exposure is extremely high near the fibre tip and reduces much faster due to expansion of the beam. Additionally, the beam profile at the fibre tip is Gaussian, rather than a flat top distribution. Therefore, the model presented in Chapter 2 does not correctly simulate light emitting from these fibres. However, if simplifications are made the radiant exposure, and therefore temperature, from these fibres can be estimated.

Using a simple geometric approach, the energy density at different distances can be estimated. If an initial spot size of diameter 8 µm\(^7\) is assumed at the fibre tip with an NA of 0.13, the expansion of the beam can be estimated at increasing distances. For a medium with a refractive index of \(n = 1.33\), at \(z = 100 \mu m\) the spot size increases to a diameter of 28 µm, 57 µm at \(z = 250 \mu m\) and 106 µm at \(z = 500 \mu m\). With a pulse length of 100 µs and peak power of 150 mW, this implies radiant exposures of 2495 mJ.cm⁻², 584 mJ.cm⁻² and 169 mJ.cm⁻² at respective distances of \(z = 100 \mu m\), \(z = 250 \mu m\) and \(z = 500 \mu m\) if no absorption is assumed to occur. By using Equations 2.14, 2.15 and the radiant exposure, the temperature at these distances can be estimated. At \(z = 100 \mu m\) a temperature of 12.0 °C is found, greater than the thresholds suggested by much of the INS literature (Izzo et al., 2007c). However, at just \(z = 250 \mu m\), this temperature is reduced to 1.90 °C. At a distance of \(z = 500 \mu m\), the temperature is only 0.286 °C, although this is still greater than the thresholds suggested in the literature.

An attempt to stimulate a guinea pig cochlea was made at the Bionics Institute (BI). With a cleaved fibre aimed towards Rosenthal’s canal, no clear response could

\(^6\)For example, Izzo et al. (2007c) used a 1850 – 1870 nm laser with maximum CW power of 1.4 W
\(^7\)8 µm was selected as it is the mode field diameter for this wavelength in the fibre used (SMF-28).
be observed at any pulse duration or laser power. Full details of this testing are provided in the following chapter, Section 6.1.1.

5.1.2 MK II: 1870 nm laser

Following the null response of the 1450 nm laser, it was decided to develop a laser with a wavelength closer to that used in the literature, despite the 1450 nm wavelength range apparently providing similar performance attributes. Finding a diode which met the power and wavelength requirements was challenging, as most diodes in the wavelength range either offered inadequate power or had a long lead-time for delivery. In the end a diode providing up to 1 W was selected (m2k Laser, m2k_BA_1850_SE). Unlike the 1450 nm laser, only a bare diode was delivered, meaning that a coupling system had to be developed. To make use of the laser, a coupling system and laser driver was manufactured together with a commercial partner (OptoTech, Australia).

Coupling all of the light emitted by the diode proved challenging, for a range of reasons. Most of the optics available were for wavelengths near 1550 nm and had different focusing distances at 1850 nm; additionally, they did not have anti-reflection coatings, leading to greater losses through Fresnel reflection. The shape of the diode (1 mm × 150 µm) made it hard to capture all the irradiation, especially as there was a strong beam divergence. To obtain the maximum power, a 600 µm diameter core fibre was used to maximise the light capturing cross section. The wavelength of this diode was longer than desired: at 50% power, a wavelength of ∼ 1870 nm was emitted at a diode temperature of 15 ºC (Fig. 5.3). At full power, this increased to ∼ 1900 nm, which corresponds to a much greater water absorption.

The current of the laser diode was set by a digital RS-232 interface, which allowed the diode current and temperature to be monitored. Pulse duration and period were controlled by a TTL input, which drove the laser diode. This was typically performed with an external signal generator.

Despite the longer than desired wavelength output from this source, an experiment was performed in a guinea pig cochlea to assess its potential for INS. Although the maximum radiant exposure at the fibre tip is low (13.4 mJ.cm⁻² for a 100 µs pulse), it is still greater than that used to generate a response by Richter et al. (2008) and other studies (Fig 5.1). The surgery and experimental arrangement was similar to that used for the 1450 nm laser experiments. Again, no response was observed, even when the fibre was positioned against the osseous spiral laminar to minimise the distance between the fibre and target neurons. Full details of the experiment are provided in Section 6.1.2.

To estimate the effect that the wavelength range of this laser diode had on the
resultant absorption in the target neurons, the spatial model (discussed in Chapter 2 was applied to estimate how the wavelength shift influenced the final temperature increase. Figure 5.4a shows the change in temperature over the wavelength range 1870 – 1900 nm, for a radiant exposure of 13.4 mJ.cm\(^{-2}\). A \(\varphi_{\text{core}} = 200 \mu m\) fibre was used for simplicity, but as the \(z_T\) distance is greater than 500 \(\mu m\) the results should also apply to an increased core diameter. When the fibre is positioned 500 \(\mu m\) away from the nerve layer, there is a decrease in the temperature in the nerve layer as the wavelength increases from 1870 nm. The 250 \(\mu m\) separation case shows a peak temperature at 1885 nm. Figure 5.4b takes this wavelength dependant change in temperature and applies it to the change in wavelength as the laser power is increased. The laser is assumed to have a constant wavelength of 1870 nm below 50% power and a linear increase to 1900 nm at 100% power. With the fibre is positioned 500 \(\mu m\) away from the nerve layer, a maximum temperature increase is found at 66% power. The 250 \(\mu m\) case is less affected by the shift in wavelength, but still has a maximum temperature increase below 100% at 85% power.

This reduction in maximum temperature change may have partially contributed to the lack of response seen. However, this reduction does not explain the lack of response when the fibre was positioned against the osseous spiral laminar, as the distance between fibre emitter and neurons would be 250 \(\mu m\) or less.

5.1.3 MK III: 1850 nm laser

To more closely replicate previous laser sources which successfully stimulated the cochlea, a replacement diode with a shorter wavelength was sourced from m2k laser,
Figure 5.4: a) Modelled temperature rise in the nerve layer for a radiant exposure of 13.4 mJ.cm$^{-2}$ from a $\varphi_{\text{core}} = 200 \ \mu$m fibre, at distances of 250 \mu m and 500 \mu m. This is equivalent to the temperatures expected for laser MK II. b) Temperature rise predicted at different power levels of the laser, when taking the varying wavelength into account.

the spectrum is shown in Figure 5.5. The wavelength is blue-shifted by approximately 20 nm compared to the initial diode, with a minimum wavelength of 1850 nm at low power and 1875 nm at full power. Additionally, OptoTech improved the coupling system, allowing 360 mW to be coupled into a 200 \mu m core diameter fibre. This improvement gave a factor of 7.6 times increase in the radiant exposure compared to laser MK II. In addition the shorter wavelength reduced the absorption at the maximum power compared to MK II, allowing for a larger temperature increase at greater depths. The same driving unit as in laser MK II was used here, with RS-232 digital control over the diode current and TTL driven pulses. The driving unit and laser unit are shown in Figure 5.6.

Figure 5.7 shows the near linear relationship between the laser current setting and the resultant power. Current was set using the digital interface, with a value of 0 – 950, with 950 corresponding to a diode current of roughly 4 A. The threshold current is near 100 and the laser power increases to 368 mW at the current setting of 950. Figures 5.8 and 5.9 show the response at different current settings and pulse lengths. While the overall pulse is close to a square wave, there is a significant ripple, roughly 15% of the total amplitude visible in the response at powers greater than 25%.

Figure 5.10a shows the change in the temperature over the wavelength range covered by the MK III laser, assuming a constant radiant exposure of 115 mJ.cm$^{-2}$. The change in wavelength for different power output is subsequently taken into account in Figure 5.10b, which unlike the MK II, shows a continuous increase in
5.1. Laser sources

Figure 5.5: Spectrum at 25%, 50%, 75% and 100% power for the diode used in laser MK III, from the datasheet provided by m2kLaser.

Figure 5.6: Picture of packaged laser MK III, showing the driving unit and diode unit.

Figure 5.7: Relationship between current setting and power output for MK III laser.
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Figure 5.8: Measurement of 100 µs duration pulses of MK III laser with current settings of I=950, 500 and 300, corresponding to power levels of 100%, 50% and 25% using a Thorlabs DET10D biased photodiode.

Figure 5.9: Measurement of 10 µs, 100 µs and 1 ms duration pulses at 100% power of MK III laser using a Thorlabs DET10D biased photodiode. The amplitude of the pulses have been scaled individually and cannot be directly compared.
the predicted temperature as the power of the laser is increased. The peak radiant exposure of 102 mJ.cm\(^{-2}\) for a 100 \(\mu\)s duration pulse, compares well to the thresholds published (e.g. 6.18 mJ.cm\(^{-2}\) (Izzo et al., 2007c)), and those shown in Figure 5.1.

The ability of this laser to generate an acoustic click was tested, as previous work with lasers intended for INS have shown a click in a humid atmosphere (Teudt et al., 2011). The emitting optical fibre was positioned within 4 mm of a the centre of the microphone. With the laser generating 360 mW, a 200 \(\mu\)s pulse duration giving a radiant exposure of 229 mJ.cm\(^{-2}\), the trace on the digital oscilloscope was suggestive of a weak acoustic response.

An experiment was performed to stimulate a guinea pig cochlea with this laser. The procedure was the same as the previous experiments (MK I and MK II). Again, no response was observed for any pulse duration or power level, even with the fibre positioned against the osseous spiral laminar. Coupling the fibre into the patchcord used to deliver the light to the cochlea, caused a loss of \(\sim 35\%\). Even taking this loss into account, the radiant exposure delivered was still \(\sim 10\) times greater than the published thresholds, as shown in Figure 5.1. The reason for this null-result is unknown, especially as the irradiance appears to be well over the thresholds levels in the published literature. A full description of the experiment is found in Section 6.1.3.
5.1.4 MK IV: 1550 nm laser

Given the limited availability of diodes in the 1850 – 1870 nm range with adequate power, an new laser using a wavelength of 1550 nm was developed by OptoTech. The 1550 nm wavelength has a similar absorption coefficient in water to 1850 nm, but it is commonly used in telecommunications and there are many more sources available in the 1550 nm region. A pigtailed diode was sourced at a wavelength of 1550 nm, coupled into a 105 $\mu$m core diameter fibre. The diode provides up to 3 W, almost a 10 times increase on the previous version of the laser (MK III). When the smaller core diameter is taken into account, this gives a 34 times increase in the radiant exposure. If coupled into a 200 $\mu$m core diameter fibre, to increase the volume of neural tissue exposed, this still gives an 9.4 times increase in radiant exposure over the previous laser.

Unlike the previous laser, the MK IV laser was updated to allow for control of current, pulse duration and pulse period on the front panel, shown in Figure 5.11. The pulse period is additionally controllable through the use of an external trigger. The laser power is controlled by adjusting the current delivered to the diode. The current is controlled by a dial on the front of the laser, which goes from 0 – 12. Figure 5.12 shows the relationship between the current setting and laser output power. As the laser was still a prototype at the point of testing, it had not been specifically adjusted to provide a linear relationship between the current setting and the resulting output optical energy.

Figure 5.13a shows the modelled increase in temperature achieved from a pulse of light when the MK IV laser is coupled into a 200 $\mu$m core diameter fibre. This gives a radiant exposure of 955 mJ.cm$^{-2}$. With this radiant exposure, a much higher temperature change is observed, 1.34 °C at 500 $\mu$m and 1.75 °C at 250 $\mu$m. Figure 5.13b gives the predicted temperature change achieved at different power levels. In
5.1. Laser sources

Figure 5.12: Relationship between current setting and power output, with linear line of best fit, for the MK IV laser.

In this case, the change in wavelength was not considered, as it was not specified by the diode manufacturer. Furthermore, at this wavelength there is less change in absorption with varying wavelength, compared to the 1850 – 1900 nm wavelength range.

To date, this laser remains untested for INS in the cochlea. As the Aculight Renoir laser (described below), was generously loaned by Dr Richter of Northwestern University, the latter was instead used for further INS experiments in the cochlea, as it is considered to be a “gold standard”. The 1550 nm has subsequently remained untested in the cochlea, partly due to a lack of time and also to an equipment failure which occurred just before a planned experiment. However, it has been successfully used for in vitro experiments with rat auditory neurons (Brown, 2013).

5.1.5 Aculight Renoir laser

Due to the failure of lasers MK I – III to generate any response in the cochlea, an Aculight Renoir was borrowed from Dr Richter’s laboratory at Northwestern University. This integrated source, has a diode laser with an adjustable wavelength of 1869 – 1889 nm and an SMA socket for fibre coupling. The laser delivers a peak power of up to 3 W, however it has a maximum duty cycle of 10% and only couples 1.5 W (peak) into a 200 μm core diameter fibre. Power is set as a percentage of maximum (100%), with the pulse length and frequency controlled by dials on the front of the unit (Figure 5.14). The laser allows pulse durations of from 10 μs to 20 ms at repetition rates between 1 Hz and 200 Hz and also allows for external triggering. Maximum power is obtained at a wavelength of 1869 nm (1.52 W) and
Figure 5.13: a) Modelled temperature rise in the nerve layer for a radiant exposure of 955 mJ cm\(^{-2}\) from a core = 200 µm fibre, at distances of 250 µm and 500 µm, over the wavelength range of λ = 1500 – 1600 nm. b) Temperature rise predicted at different power levels of the MK IV laser.

Figure 5.14: Picture showing the front panel of the Aculight Renoir Laser.

reduces linearly with wavelength to 1.12 W at 1889 nm. The measured change in output power is shown in Figure 5.15, for a core = 200 µm core diameter optical fibre.

Unlike the other lasers used in this wavelength range, no power dependent shift in wavelength was documented. The modelled change in temperature over the wavelength range provided by the laser is shown in Figure 5.16a. When the fibre is located 250 µm from the nerve, there is minimal change in temperature over the wavelength range. At the greater distance of 500 µm, 1870 nm gives the greatest temperature increase, with the temperature reducing at longer wavelengths. When the change in power over the wavelength range (shown in Fig. 5.15b) is taken into account, there is a drop in temperature at longer wavelengths, especially for greater
5.1. Laser sources

Figure 5.15: Change in peak power output when adjusting peak power and wavelength on the Aculight laser.

separation between the fibre and target neurons.

Measurements of the pulse shapes from the Aculight laser are shown in Figures 5.17 and 5.18. This output from a 200 µm core diameter fibre was aimed at a Thorlabs DET10D biased photodiode. The photodiode was separated by sufficient distance to prevent saturation at 100% power. The output of the photodiode was connect to a Tektronix TDS 3054 digital oscilloscope (DSO), which was used for data acquisition. The DSO averaged 64 times and data was run through a low pass filter ($f_c = 2$ MHz) for presentation purposes. Figure 5.17 shows the response to a 100 µs duration pulse at 100%, 50% and 25% of maximum power. The pulse shape shows good correlation to the trigger pulse, with only a small overshoot at 100%. At lower powers, there is a slight delay in the response ($\sim 10$ µs), shortening the total pulse duration slightly. Figure 5.18 shows the response for 10 µs, 100 µs and 1 ms duration pulses. The delay in turning on can clearly be seen for the 10 µs duration pulse, with it taking $\sim 3$ µs to reach 63% of the maximum amplitude. Overall, a good correlation was observed between the trigger pulse and signal on the photodiode.

As lasers used for INS have previously been observed to generate an acoustic click in water and a humid atmosphere (Teudt et al., 2011), the potential of this aculight laser to generate a click was tested. Figure 5.19 shows the acoustic response to a 200 µs pulse with an energy of 260 µJ (radiant exposure of 0.8 J.cm$^{-2}$), with the fibre oriented parallel to the microphone membrane and $\sim 4$ mm from the centre of the microphone membrane (G.R.A.S Type 46AE microphone, G.R.A.S Type 26CA preamplifier, G.R.A.S Type 12AL CCP Power Supply). This microphone has a frequency response of 3.15 Hz – 20 kHz ($\pm 2$ dB) and nominal sensitivity of 50 mV.Pa$^{-1}$. The microphone detected a spike at the beginning and end of the laser
Chapter 5. Experimental Materials and Methods

Figure 5.16: a) Modelled temperature rise in the nerve layer for a radiant exposure of 446 mJ cm\(^{-2}\) from a \(a_{\text{core}} = 200 \, \mu\text{m}\) fibre, at distances of 250 \(\mu\text{m}\) and 500 \(\mu\text{m}\). b) Temperature rise predicted at different wavelengths, taking into account the varying absorption due to wavelength and changes in maximum power levels.

Figure 5.17: Measurement of 100 \(\mu\text{s}\) duration pulses at 100\%, 50\% and 25\% power using a Thorlabs DET10D biased photodiode.
Figure 5.18: Measurements of 10 µs, 100 µs and 1 ms duration pulses at 100% power using a Thorlabs DET10D biased photodiode. The pulse amplitudes have been scaled individually and cannot be directly compared.

Figure 5.19: Acoustic click generated by the Aculight Renoir laser for a 200 µs duration pulse at 100% power.
pulse, similar to that observed by Teudt et al. (2011). Taking the peak-peak intensity recorded of 0.49 mV (Fig 5.19), this corresponds to a peak pressure of approximately 9.8 mPa or 54 dB SPL. When the fibre was disconnected from the laser, no sound was recorded. As concluded by Teudt et al. (2011), it is likely this acoustic event is due to thermal expansion of water.

Given the difficulty in achieving optical stimulation of neurons with lasers I – III, an experimental protocol was developed in consultation with Dr Richter of Northwestern University, and is described in Section 5.2.5.

5.2 Experimental Protocol for INS in the cochlea

5.2.1 Animal Model

Eight \((n = 8)\) young adult male and female pigmented guinea pigs were used to collect data for the experimental studies included in this thesis. Four were used for the studies with lasers MK I, MK II and MK III, while four where used for studies with the Aculight laser. The experimental cohorts are summarised in Table 5.2. All acutely deafened animals were first tested as control normal hearing animals and all chronically deafened animals had both cochleae tested.

All experimental procedures involving animals were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and with the Guidelines laid down by the National Institutes of Health in the US regarding the care and use of animals for experimental procedures and were approved by the Royal Victorian Eye and Ear Hospital Animal Research and Ethics Committee.

The protocol described below is derived from the Bionics Institute’s standard operating procedures (SOPs)\(^8\) and ethics application for this project. All animal preparation was conducted by A. Wise, J. Fallon, S. Irving and/or R. Shepherd of the Bionics Institute as the competent persons with experience in these procedures. The author’s contributions was confined to preparation and operation of the electrical and optical equipment required to perform the INS experiments. However, for completeness, the animal experimental procedures are summarised in the following sections.

\(^8\)The Royal Victorian Eye & Ear Hospital: Animal Research & Ethics Committee: Standard operating procedures: No 3, Guinea pig deafening, using kanamycin and frusemide; No 4, Inhalant anaesthesia - masking down with isoflurane; No 7, Protocol for auditory brainstem response recording and electrically evoked brainstem response recording; No 11, Guinea pigs - anaesthesia, pre- and post-operative care.
### 5.2. Experimental Protocol for INS in the Cochlea

#### Table 5.2: Experimental cohorts for the different INS lasers and deafening techniques.

<table>
<thead>
<tr>
<th></th>
<th>MK I</th>
<th>MK II</th>
<th>MK III</th>
<th>MK IV</th>
<th>Aculight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>1450</td>
<td>1870 – 1900</td>
<td>1850 – 1870</td>
<td>1550</td>
<td>1869 – 1889</td>
</tr>
<tr>
<td>Acutely Deafened</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Chronically Deafened</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 ( (n_{\text{cochlea}} = 4) )</td>
</tr>
</tbody>
</table>

#### 5.2.2 Deafening procedures

As previously discussed in Section 1.2.1, to prevent hair cell mediated electrophonic (Shepherd and Javel, 1997) or optoacoustic (Teudt et al., 2011) activity from contaminating the auditory brainstem response (ABR), animals were chemically deafened. Deafening was either acute, at the time of the experiment, or chronic, typically four weeks before the experiment. Acute deafening results in just the hair cells being killed, while chronic deafening causes a reduction in the spiral ganglion neuron population in the cochlea. The two deafening techniques used are described in the following sections.

**Acute Deafening**

Acute deafening was performed by gently aspirating the ototoxic aminoglycoside, neomycin sulfate, though the cochlea during the following procedure. After first anaesthetising the animal, exposing the cochlea and performing the cochleostomy (see 5.2.3 Surgical Procedure), the oval window was opened by removal of the stapes footplate and the perilymph was gently aspirated. A polyurethane cannula was inserted into the scala tympani through the cochleostomy and neomycin sulfate \((\sim 2.5 \text{ ml of } 10 \text{ mg.ml}^{-1} \text{ solution in sterile saline})\) was slowly perfused through the cochlea, with gentle aspiration at the oval window (Hardie and Shepherd, 1999). The perfusion procedure was repeated at least three times to ensure thorough ototoxin exposure.

**Chronic Deafening**

The chronic deafening procedure was a multistep process. One week before ototoxic deafening was performed, the hearing status of the animals was assessed using a click stimuli and recording of acoustic ABRs. The animals were anaesthetised with an intramuscular delivery of ketamine \((60 \text{ mg.kg}^{-1})\) and xylazine \((4 \text{ mg.kg}^{-1})\) while undergoing this testing.

Chronic deafening was subsequently performed four weeks before experimentation using a combination of Kanamycin (an ototoxic aminoglycoside) and Frusemide,
which has previously been shown to produce consistent symmetric bilateral deafness (Gillespie et al., 2003; Shepherd et al., 2008; Landry et al., 2011). Each animal was anaesthetised with gaseous isoflurane, using a 4% mixture with air for induction in an induction box. As soon as loss of muscle tone was observed, the animal was removed from the induction box and maintained on 1.5 – 2% isoflurane with 1 – 1.5 l.min\(^{-1}\) oxygen via a face mask. The neck was shaved and cleaned using antiseptic, after which the jugular vein was exposed using aseptic surgical techniques. A cannula was inserted into the vein and warmed Frusemide (130 mg.kg\(^{-1}\)) diluted 1:2 in Hartmann’s solution was slowly infused into the vein over several minutes. The cannula was then removed, the vein tied off and the wound closed with cyanoacrylate adhesive. Kanamycin (420 mg.kg\(^{-1}\)) is then administered subcutaneously in 2.5 – 3 ml of warmed Hartmann’s solution. The animal was kept on oxygen until showing signs of recovery and was transferred to a recovery box once able to sit up on its own. The animal was then carefully monitored over the next week.

One week after deafening, each animal was anaesthetised with an intramuscular delivery of ketamine (60 mg.kg\(^{-1}\)) and xylazine (4 mg.kg\(^{-1}\)). The hearing status was re-assessed using a click stimuli. The animal was defined as profoundly deaf if the hearing threshold increased by \( \geq 50 \text{ dB} \). All animals used in this study were shown to be profoundly deaf.

### 5.2.3 Surgical Procedure

Anaesthesia was induced with an intramuscular delivery of ketamine (60 mg.kg\(^{-1}\)) and xylazine (4 mg.kg\(^{-1}\)). Anaesthesia was maintained with top-up doses of ketamine (40 mg.kg\(^{-1}\)) and xylazine (4 mg.kg\(^{-1}\)), administered at one third to one sixth of the induction volume every 40 – 50 min, or if a periodic toe pinch withdrawal reflex test demonstrated insufficient anaesthesia. The animal was kept on a thermostatic heating pad at 38 °C to maintain body temperature in the normal range.

Stainless steel recording electrodes were then inserted (as described in the following section) and the animal positioned 10 cm away from a speaker to assess the pre-operative hearing status using a click stimuli and ABR recording.

Animals were prepared for surgery by shaving the area near the neck and skull and then injecting a local anaesthetic (lignocaine, 0.1 ml) at the incision site. A post-auricular incision was made and the temporalis muscle retracted, exposing the tympanic bulla. The dorsal region of the bulla was drilled with a 2 mm cutting burr to expose the cochlea. A cochleostomy was drilled into the basal turn using a diamond burr to thin the cochlea wall. After clearing the bone debris, the endosteum was perforated to expose the scala tympani and the modiolus.
5.2. Experimental Protocol for INS in the cochlea

Figure 5.20: Example of guinea pig acoustic ABR recording from free field click acoustic stimulus (average from 200 trials). Figure adapted from Landry et al. (2011), copyright Elsevier and is used with permission.

A platinum ball electrode was inserted through the cochleostomy to first assess cochlea function. An optical fibre connected to the laser of interest was then inserted through the cochleostomy and aimed towards the spiral ganglion neurons in Rosenthal’s Canal. In some animals a cochleostomy was performed in a more apical turn to assess whether the spiral ganglion neuron population at this location responded differently to the basal turn.

At the conclusion of the experiment, the animals were sacrificed with an overdose of anaesthetic sodium pentobarbital (150 mg.kg\(^{-1}\)) and intracardially perfused with formalin fixative. Tissues were collected to allow histological analysis.

5.2.4 Evoked Auditory Brainstem Recordings

The response in the cochlea and auditory system was assessed by recording the Auditory Brainstem Response (ABR) to the various stimuli; acoustic (AABR), electrical (EABR) and optical (OABR), using standard techniques (Coco et al., 2007; Fallon et al., 2009; Landry et al., 2011). These recording were performed in an electrically and acoustically insulated Faraday room. Three stainless steel electrodes were inserted in the animal, which differentially recorded the signal. The electrodes used were vertex positive, neck negative and thorax ground.

ABRs were averaged across 100 trials, presented at a rate of 20 Hz, and at least two sets of recordings were obtained at each stimulus intensity level. ABRs were recorded with Igor Pro software (Wavemetrics, USA) via a NI USB-6251 data acquisition device (National Instruments, USA) (100 kHz sampling). After acquisition
the first $\sim 1$ ms of the signal was discarded to remove any electrical artefact\(^9\) and the resultant waveform was digitally filtered with a 300 – 3000 Hz bandpass filter (Butterworth, second order). ABR thresholds were visually determined by a 200 $\mu$V increase of the PIII-NIII wave in two separate recordings. The PIII-NIII wave has a typical latency of 3 – 4 ms in a guinea pig, depending upon the stimulus type and intensity. The response was determined by finding the waveform amplitude in the 2 – 5 ms window, selected to capture the PIII-NIII wave. This window is wider than required to capture the PIII-NIII wave, as the exact response amplitude of the PIII-NIII wave was not critical to the results, instead the focus was on detecting a significant event above the noise floor. As such, the simplicity of a wider window was preferred over using an exact window. This window was varied when using a delayed stimulus. Figure 5.20 shows an example of an acoustic ABR recording in a guinea pig with the different waves marked (Landry et al., 2011).

Electrical stimuli were presented with single biphasic anodic leading phase square current pulses, either 25 $\mu$s/phase with 8 $\mu$s interphase gap, or 100 $\mu$s/phase with 50 $\mu$s interphase gap. All electrical stimulation was monopolar. Although bipolar and phased array stimulation provide more localised excitation, electrical monopolar stimulation is used in the great majority of current cochlear implant patients (Seligman and Shepherd, 2004). When using combined electrical and optical stimulation, or hybrid electro-optical stimulation, the electrical pulse was delayed to end at the same time as the optical pulse, as this has been shown to be most effective (Duke et al., 2012a).

### 5.2.5 Systematic Protocol for INS studies

Due to the failure of lasers MK I, MK II and MK III to evoke a response to INS in the cochlea, a protocol was developed with the Richter group at Northwestern University to assist in reproducing their results. The protocol consists of three stages with an example of the results previously obtained are shown in Figure 5.21. The protocol consists of three stages, as shown in the three columns: The first stage is before the cochlea is been opened; the second is after opening the cochlea and before deafening; the third stage is after deafening the opened cochlea. First, the optical fibre is positioned next to the undamaged cochlea, without directing the light towards it. The sound generated by the laser pulse (Fig 5.19) can be detected by the middle and inner ear and will generate a neural response. This is shown in the first column of Fig 5.21, along with the response to a pure acoustic stimulus. The cochlea is then opened by performing a cochleostomy to allow the optical fibre access to the

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\(^9\)The length of the discarded signal was increased when the stimulus was delayed
spiral ganglion neurons. Column two, row one of Figure 5.21 shows the response in the cochlea from the laser directed directly at the neurons. However, when the fibre is positioned next to the cochlea (column two, row two), no response is observed due to hearing loss from damage to the cochlea (column two, row three). The cochlea is then deafened with neomycin and hearing thresholds are further elevated (column three, row three). When the fibre is placed next to the cochlea, no acoustic response is seen, while when the fibre is inserted through the cochleostomy towards the spiral ganglion neurons a response is observed.

This protocol was designed to allow for simple replication of INS in the cochlea and makes use of the acoustic effects of infrared pulses observed by Teudt et al. (2011). In the experiments described in Section 6.2, two changes were made compared to the experiments described: ABR responses rather than CAPs were recorded and the deafening protocol included aspiration of neomycin through the cochlea, rather than allowing it to perfuse through the cochlea. ABRs were used as the indicator of response as it is the standard technique used by the Bionics Institute (Coco et al., 2007; Landry et al., 2011). Neomycin was aspirated through the cochlea as this is the standard technique at the Bionics Institute to ensure that animals are profoundly deaf in an acute preparation.
Figure 5.21: Matrix of different experimental conditions and the results (Richter, 2013). This matrix formed the basis of the protocol for acutely deafened guinea pig experiments. Row one shows the response when the fibre is directed towards the spiral ganglion neurons through a cochleostomy. Row two shows the response when the fibre is position next to, but not aimed at, the cochlea. Row three shows the acoustically generated CAP responses. Column one shows results from before the cochlea is opened, column two after performing a cochleostomy and column three after deafening with neomycin. The figure was provided by Dr Richter.
This chapter describes infrared stimulation experiments performed with the lasers discussed in Chapter 5. Experimental results have been broken up into three sections: Section 6.1 discusses the experiments performed with lasers developed in collaboration with OptoTech (lasers MK I, MK II and MK III); Results from the Aculight Renoir laser are presented in Section 6.2; Section 6.3 explores the potential of hybrid electrical-optical stimulation to reduce optical thresholds in the cochlea. Section 6.4 summarises the results and discusses possible mechanisms behind the results observed in the context of previously published literature.

6.1 Initial Experiments

This section describes the initial infrared stimulation experiments performed with laser sources MK I, MK II and MK III, developed in collaboration with OptoTech.

6.1.1 Laser MK I

Using the 1450 nm laser (MK I, described in Section 5.1.1), an attempt was made to stimulate the guinea pig cochlea with INS. After exposing the cochlea and performing
a cochleostomy, the cochlea was deafened by aspirating neomycin. This procedure was repeated six times to ensure the cochlea was profoundly deaf. A cleaved single mode fibre was inserted into the cochleostomy and aimed towards the spiral ganglion neurons in Rosenthal’s canal. Pulse durations of 100 µs to 1 ms were used, at a repetition rate of 10 Hz, with a peak power of 100 mW\(^1\). An example of an ABR recording with a 1 ms pulse is shown in Figure 6.1a, where the two (or more) recordings at each stimulus intensity are overlapped. The response amplitude for the 2 – 5 ms window as discussed in Section 5.2.4 shown in Figure 6.1b. Laser energy is given as energy per pulse, as radiant exposure is not a meaningful measure of intensity with a single mode fibre (as discussed in Section 5.1.1). Noting that the waveform at 2 ms is an artefact from the trigger, no clear response to the infrared laser in the ABR can be observed.

After ABR recordings showed no clear response, the laser was checked to confirm that triggering was working and that the pulse energy delivered was as expected. As the total power output of this laser is lower than that of other laser sources, pulse durations of up to 10 ms were attempted, in the hope that longer pulses may elicit a neural response. When no response was again observed, the fibre position was adjusted both into more peripheral and central locations. In total, four positions were attempted. A final attempt at stimulation was made by inserting the fibre through a hole made in the osseous spiral lamina. This was performed to reduce the distance between the fibre and the neurons, to maximise the radiant exposure that the neurons were exposed to. Again, no clear response was observed. At the

\(^1\)Approximately 2 dB or 33% of laser power was lost in optical fibre coupling.
6.1. Initial Experiments

Figure 6.2: Histology of surgery and fibre positioning, area targeted by the optical fibre was damaged at the end of the experiment, allowing the targeted area to easily be identified in histology. Black oval highlights the spiral ganglion neuron location and arrow shows approximate fibre position. a) Shows the cochleostomy and cochlea, b) shows a zoomed in region showing the spiral ganglion neurons. Figure provided by A. Wise of the Bionics Institute.

As previous work on INS in the cochlea emphasises that spiral ganglion neurons are the target of infrared stimulation and that the laser light must be incident on them (Moreno et al., 2011). The importance of correctly targeting the SGNs has also been emphasised (Richter, 2013). To confirm that the fibre was positioned correctly and targeting the SGNs, an area under the fibre was manually damaged at the end of the experiment with laser MK I, to mark the area for histology. Figure 6.2a shows the cochleostomy into the scala tympani, in the basal turn of the cochlea. Figure 6.2b shows a zoomed in section, showing the damage to the osseous spiral lamina in front of the spiral ganglion neurons. This confirmed the accurate positioning of the fibre and that the spiral ganglion neurons were directly exposed to the infrared light emitted by the fibre.

The reason for this null result is unknown. Although the radiant exposure at distance of up to 500 µm is comparable to previously published thresholds and therefore should be adequate to allow for INS in the cochlea. At a distance of 500 µm the spot size is roughly half the diameter compared to a 200 µm core diameter and it is possible that as the area exposed did not excite a large enough population of neurons to generate a measurable ABR response.
6.1.2 Laser MK II

After no response was observed with MK I laser, a new laser closer to the wavelength range previously used for INS in the cochlea was developed. This laser is described in Section 5.1.2. An experiment was performed in a single guinea pig to evaluate this laser’s ability to stimulate the cochlea through INS. Unlike the previous experiment, the cochlea was not deafened to give the maximum chance of observing a response. After exposing the cochlea and performing a cochleostomy, an electrode was inserted inside the cochlea to confirm that the cochlea and recording equipment was functioning. The optical fibre was then inserted and aimed at the spiral ganglion neurons. Stimulation was performed at a rate of 10 Hz, with 100 repetitions. For pulse lengths of 100 µs to 2 ms and corresponding maximum radiant exposures of 13.4 mJ.cm$^{-2}$ to 268 mJ.cm$^{-2}$, no clear response was observed.

An example of an ABR recording due to a 1 ms INS pulse is shown in Figure 6.3a. Figure 6.3b shows the response to a control click stimulus, confirming that the auditory system and recording was functioning after the cochlea was exposed and cochleostomy performed. Again, a number of different positions for stimulation were attempted, with no clear response at any location. Due to the larger fibre diameter ($\varnothing_{\text{core}} = 600 \, \mu\text{m}$) no attempt was made to make a hole in the osseous spiral lamina, although the fibre was positioned so that it was touching the osseous spiral lamina.

At this laser’s maximum power, the radiant exposure was $\sim 2$ times greater than reported thresholds for INS in the cochlea (e.g. Richter et al. (2008)). Therefore, it is possible that the lack of a response with the MK II laser is in part due to challenges.
in getting a response near threshold without optimisation. However, the challenges of fibre position optimisation should have been reduced by the large core diameter fibre irradiating a greater area of tissue. In addition four different optical fibre positions were attempted. Another possible explanation is that the shift in wavelength at higher powers, discussed in Section 5.1.2 and shown in Figures 5.3, could result in more energy being absorbed in the perilymph before it reaches the neurons. The predicted change in temperature by the model is shown in Figure 5.4. The problem of perilymph absorption was reduced by positioning the fibre against the osseous spiral lamina in some fibre positions, reducing the distance between the fibre and nerves to less than 250 $\mu$m, which should not see a decrease in energy absorbed at maximum power (as shown in Figure 5.4).

6.1.3 Laser MK III

The wavelength, power levels and fibre properties of laser MK III more closely matched those used in the literature. Laser MK III provided approximately up to 13 times the radiant exposure of published thresholds for acutely deafened animals. For example Richter et al. (2008) gives a threshold of 7 mJ.cm$^{-2}$ for a 100 $\mu$s pulse, and the maximum radiant exposure was above that reported for INS in other targets (Figure 5.1). Additionally, laser MK III used an optical fibre with properties ($\varnothing_{\text{core}} = 200 \mu$m, NA = 0.22) matching those commonly found in the cochlea INS literature. To increase the probability of a successful outcome with this laser, two guinea pigs were used. As with the experiment with laser MK II, the experiments were performed in normal hearing animals, to maximise the chance of a response being observed.

Figure 6.4a shows the response to infrared stimulation with a 200 $\mu$s pulse duration, pulse energy of up to 68 $\mu$J and radiant exposure of up to 215 mJ.cm$^{-2}$. No clear response can be seen and this was typical of attempts to stimulate the cochlea with this laser. In both animals, four or more different fibre positions were attempted, including a final attempt where a hole was made in the osseous spiral lamina to position the fibre closer to the neurons. Figure 6.4b shows the typical response to a platinum ball electrode inside the cochleostomy, stimulating with 25 $\mu$s per phase. The electrical stimulation confirmed that the cochlea was functioning normally in both cases.
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Figure 6.4: a) Typical ABR recording from the MK III laser; here a 200 µs pulse at radiant exposures of 0 – 215 mJ.cm\(^{-2}\) was used. No clear response was observed. b) Typical response to an electrical stimulus. Note that different scales are used between a) and b).

6.2 Aculight Laser Experiments

Due to the difficulties in observing a laser evoked response in the cochlea with the lasers discussed in the previous section, an Aculight laser was borrowed from the Northwestern group’s laboratory and an experimental protocol for INS in acutely deafened animals was developed. The protocol is discussed in Section 5.2.5. Use of the Aculight laser was aimed at reducing the differences between our experimental conditions and those of the Northwestern group.

Typically, \(\sim 1\) dB or 20\% of the power was lost when coupling the fibre to the laser, leaving a usable maximum power of 1.2 W. Unless otherwise stated, a wavelength of 1869 nm was used.

6.2.1 Acutely Deafened Experiments

As shown in Table 5.2, two guinea pigs were used for this part of the study. The results from each animal are presented separately in this section.

First Animal

The first guinea pig had its right cochlea exposed as described previously (Section 5.2.3), however the left ear was not plugged, which made changes in acoustic thresholds due to deafening difficult to determine. Figure 6.5 shows the animal’s response to an acoustic stimulus, after the right bulla had been opened and the cochlea exposed. As the Aculight laser can generate sound in a humid atmosphere or in water (Teudt
et al., 2011), the cochlear response to this sound was tested. Figure 6.6 shows the response to the fibre located next to, but not aimed at, the intact cochlea. Pulse durations of 100 µs were used. Again, a near linear response is observed in relation to an increasing stimulus. At maximum laser power, a response of ∼8.5 mV was observed.

After a cochleostomy was performed, the fibre was inserted inside the cochlea and positioned to illuminate the spiral ganglion neurons in Rosenthal’s canal. An example of the fibre positioning is shown in Figure 6.7. Pulse durations of 100 µs were used and the response to this stimulation is shown in Figure 6.8 as the black traces. With the fibre directed towards the spiral ganglion neurons the maximum response was ∼2.5 mV, which saturated before the maximum radiant exposure.

The cochlea was made profoundly deaf through use of neomycin. The neomycin was aspirated through the cochlea three times (as discussed in Section 5.2.2). The response to INS with a 100 µs pulse length is shown in red in Figure 6.8, no clear response to the laser was observed at any pulse energy. In this figure, the response at the same energy level for the control case (black) and after acute deafening (red) is overlayed. It should be noted that the acoustic click threshold did not change greatly, as the left ear was not adequately blocked in this experiment (data not shown).

After no clear response could be evoked in the profoundly deaf cochlea with a 100 µs duration pulse and pulse energies of up to 120 µJ, corresponding to radiant exposures of 380 mJ cm⁻², cochlea function was confirmed with electrical stimulation. A platinum ball electrode was inserted inside the cochleostomy with a needle return electrode. When stimulating with 25 µs per phase, an EABR threshold of ∼625 µA was found as shown in Figure 6.9. A total of five different optical fibre positions
Figure 6.6: a) ABR recording from Aculight INS laser ($t_{\text{pulse}} = 100 \ \mu s$) next to intact cochlea but not aimed towards it. b) Response from (a) plotted against the stimulus intensity.

Figure 6.7: Picture showing the approach of the fibre into the scala tympani and towards the osseous spiral lamina.
6.2. Aculight Laser Experiments

Figure 6.8: a) ABR recording from Aculight INS laser ($t_{\text{pulse}} = 100 \mu$s) with fibre aimed at Rosenthal’s canal inside the cochlea: before deafening control (black); and after acutely deafening the cochlea with neomycin (red). b) Response from (a) plotted against the laser pulse energy.

Figure 6.9: a) EABR recording from electrical stimulation after deafening the cochlea with neomycin. b) Response from (a) plotted against the laser pulse energy.
inside the cochleostomy were attempted, including moving the fibre up to the osseous spiral lamina to reduce the distance between the fibre and the nerves. To investigate whether the optical threshold were higher than expected after deafening, the cochlea was exposed to pulse lengths up to 1 ms, corresponding to a maximum energy of 1.2 mJ or radiant exposure of $3.8 \text{ J.cm}^{-2}$. No clear response was observed for any of these parameters.

**Second Animal**

To allow for the acoustic thresholds to be accurately determined in the cochlea under investigation, in the second animal the right cochlea was unilaterally deafened by opening the bulla and flushing neomycin into the cochlea three times. After deafening the right side, the left bulla was opened and an acoustic threshold taken. Unlike the previous animal, a larger opening was made. This resulted in damage to the middle ear structures causing conductive hearing loss. This gave an acoustic threshold increase of roughly 35 dB without any deafening.

The response of the cochlea to the optoacoustic click was assessed. The fibre was positioned next to, but not directed towards, the intact cochlea and exposed to 100 $\mu$s pulse duration of INS. No clear response to the laser was observed. This may be due to the middle ear damage and resultant hearing loss.

The cochlea was then opened, optical fibre inserted through the cochleostomy and towards the spiral ganglion neurons. No response could be evoked with a pulse duration of $t_{\text{pulse}} = 100 \mu$s and pulse energies of 120 $\mu$J. No acoustic response was observed in response to an acoustic click test, likely due to additional conductive losses from opening the cochlea. A platinum ball electrode placed inside the cochleostomy showed a typical electrophysiological response, with an EABR threshold of $\sim 950 \mu$A (25 $\mu$s per phase). Again, after confirming electrophysiological function, longer pulses at maximum power were attempted, all showing no clear response to stimulation.

After performing control experiments the animal was deafened. Two steps of deafening were performed in this experiment: gentle deafening, where 0.2 ml of 10% w/v neomycin was allowed to perfuse through the cochlea; and profound deafening, where 10% w/v neomycin was aspirated through the cochlea. Attempts at INS were made after both gentle and profound deafening and no clear response was observed. The cochlea still responded well to electrical stimulation at all stages of the experiment.

Overall, no clear response to infrared laser exposure was observed in a cochlea made profoundly deaf with neomycin.
6.2.2 Chronically Deafened Experiments

As no clear response was observed in any acutely deafened animals, two chronically deafened animals were examined to assess whether the deafening techniques may have interfered with the process behind INS. It has been suggested (Richter, 2013) that the absence of a response in acutely deafened animals, could be due to neomycin acting as a calcium channel blocker (Zhou et al., 1996; Canzoniero et al., 1993), which could hinder INS if calcium channels are involved (Dittami et al., 2011). This potential problem could be avoided by chronically deafening the animals, so that any of the chemical involved would no longer be present. Four weeks before the planned experiment, both animals were deafened with kanamycin and frusemide as previously described in Section 5.2.2. One week after deafening hearing was reassessed and both animals were found to be profoundly deaf. Both cochleae were tested in both animals, giving a total of four cochleae examined. Furthermore, as spiral ganglion neuron survival after deafening is typically greater in the apical turns of the cochlea (Leake and Hradek, 1988), a second cochleostomy was performed in a more apical turn on both cochleae of the second animal. Reduced spiral ganglion neuron counts have been suggested to hinder a response to INS (Richter et al., 2008). Therefore, Rosenthal’s canal was targeted in the apical region to maximise the chance of a response due to the greater density of intact neurons typically present in this region. The parameter space explored is summarised in Table 6.1.

<table>
<thead>
<tr>
<th>Animal</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cochlea</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>INS Response</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Electrical Response</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Basal Turn</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>Apical Turn</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long Pulses</td>
<td>T</td>
<td>T</td>
</tr>
</tbody>
</table>

Table 6.1: Summary of the INS experiments performed in chronically deafened animals. Basal and Apical Turn rows indicate whether INS was tested in the basal or more apical turn of the cochlea; the ‘Long Pulses’ row indicates whether pulses longer than 500 µs were tested. ‘T’ indicates that the item was investigated in that cochlea.

A typical response to INS in the chronically deafened cochlea is shown in Figure 6.10. Here a pulse duration of 100 µs was used with a pulse energy up to 120 µJ (radiant exposure 380 mJ.cm\(^{-2}\)). The fibre was aimed at the spiral ganglion neurons in Rosenthal’s canal in the basal turn of the cochlea. The null response observed here was typical of all cochleae tested with optical stimulation alone. In the second animal, which in addition to the basal turn has a more apical turn exposed to infrared
irradiation, no site, including the more apical turns, showed any response to the laser. After testing with shorter pulses of 100 µs and 500 µs, longer pulses of up to 10 ms were attempted. These were delivered at a slower rate of 2 Hz to reduce the build up of potentially damaging heat. Again, no response was observed.

As shown in Table 6.1, all cochleae tested responded to electrical stimulation. A typical response is shown in Figure 6.11. The response is much weaker than shown in the acutely deafened animals, largely due to spiral ganglion neuron death. It should be noted that the ball electrode was placed on the round window of the cochlea, rather than inside the cochleostomy as done in the acutely deafened animals. All cochleae displayed an ABR in response to electrical stimulation.

In the last cochlea, after no clear response was evoked when targeting the spiral ganglion neurons, a final attempt was made to stimulate the cochlea nerve rather than spiral ganglion neurons. Surgery was performed to create a hole in the modiolar wall and the fibre was inserted though it, targeting the cochlea nerve. Using pulse lengths of 100 µs to 1 ms at maximum laser power, no clear response was evoked.

6.3 Hybrid electrical-optical Stimulation

The combination of electrical stimulation and INS, or hybrid electrical-optical stimulation, has been shown to reduce thresholds of both stimuli (Duke et al., 2009, 2012a,b) in the rat sciatic nerve and the Aplysia buccal nerve. As no clear response

\[2nd\ turn\ tested\ in\ left\ ear,\ 3rd\ in\ right\]
Figure 6.11: a) Typical ABR recording from electrical stimulation in chronically deafened animals. Here 100 µs per phase was used with a ball electrode on the round window. b) Response from (a) plotted against stimulus current.

from INS alone was observed in either acutely or profoundly deaf animals, hybrid electrical-optical stimulation was attempted in the cochleae to assess how the two stimuli may interact. The previous work by Duke et al. (2012a) was focused on using sub-threshold electrical stimulation to reduce optical thresholds. However sub-threshold optical pulses could also be used to reduce electrical thresholds. Therefore, using hybrid electrical-optical stimulation allows for the examination of any sub-threshold response from INS, by using electrical stimulation to get close to threshold, and whether INS directly interacts with the neurons by potentially reducing the stimulus intensity required to generate a measurable response.

To examine the interaction between electrical stimulation and INS, an optical fibre was positioned inside the cochlea and aimed towards the spiral ganglion neurons. An electrode was also positioned inside or on the round window of the cochlea. The electrical pulse was usually delayed so both stimuli would finish at the same time, as this has been shown to be most efficient when using hybrid stimulation (Duke et al., 2012a) An EABR was measured without the laser on but with the optical fibre in position, over a range of currents. Then the laser was enabled and stimulation repeated over the same range. Overlaying the results with laser on and laser off allows any enhancement due to the laser to be identified.

Hybrid electrical-optical stimulation was investigated in three profoundly deaf animals after INS alone had failed to generate a clear response. One animal was acutely deafened and two were chronically deafened. Both cochleae of the chronically deafened animals were tested with hybrid stimulation. No experiments were performed in normal hearing animals. A summary of the experiments and results
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<table>
<thead>
<tr>
<th>Animal</th>
<th>A2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deafening</td>
<td>Acute</td>
<td>Chronic</td>
<td></td>
</tr>
<tr>
<td>Cochlea</td>
<td>L</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>INS Response</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Electrical Response</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Hybrid Stim Threshold Reduction</td>
<td>50 µA</td>
<td>Nil</td>
<td>25 µA</td>
</tr>
</tbody>
</table>

Table 6.2: Summary of electrical-optical hybrid stimulation experiments performed in deafened animals.

are shown in Table 6.2.

Only one control/acutely deafened animal was tested for hybrid electrical-optical stimulation. The animal used was the second, which had been exposed to two different deafening techniques. As previously discussed (Section 6.2), the deafening techniques were: gentle deafening where neomycin was allowed to naturally perfuse through the cochlea; and profound deafening where neomycin is was aspirated three times through the cochlea. An example of the response observed in a guinea pig after gentle acute deafening was performed is shown in Figure 6.12. The laser used a wavelength of 1869 nm, with pulse length of 1 ms. The electrical stimulation began at 942 µs after the optical stimulation, so they finished at the same time. A reduction in threshold of roughly ∼50 µA was observed. The fibre and electrode were then removed and thorough deafening performed. After thoroughly deafening the cochlea, an enhancement was still observed as shown in Figure 6.13. Again a roughly ∼50 µA enhancement can be seen, although the overall response is weaker due to the electrode being moved during the second deafening procedure.

Experiments in chronically deafened animals also showed a potential reduction in threshold, although any benefit was weaker. Due to the reduction in spiral ganglion neurons due to deafening, electrical stimulation used pulse durations of 100 µs per phase which gives a lower threshold current compared to the 25 µs used in the acutely deafened experiments. INS pulses were 500 µs at maximum laser power and the electrical pulse began at 250 µs so they finished at the same time. With the optical fibre positioned inside the cochleostomy and directed towards the spiral ganglion neurons, an electrode was placed on the round window of the cochlea. This placement was chosen as it was not possible to fit both the electrode and fibre together inside the cochleostomy and have room to adjust the fibre while obtaining visual confirmation of its location. An enhancement was also observed in three of the four chronically deafened cochleae. The one that did not display an enhancement

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3Electrical stimulation was 25 µs per phase, with an 8 µs interphase gap
4An interphase gap, of 50 µs was used
6.3. Hybrid electrical-optical Stimulation

Figure 6.12: a) ABR recording from electrical stimulation (black) and hybrid electrical-optical stimulation (red). INS laser with fibre aimed at Rosenthal’s canal inside the cochlea. After deafening cochlea by allowing neomycin to perfuse through cochlea (no aspiration) b) Response from (a) plotted against the stimulus intensity.

Figure 6.13: a) ABR recording from electrical stimulation (black) and hybrid electrical-optical stimulation (red). INS laser with fibre aimed at Rosenthal’s canal inside the cochlea. After thoroughly deafening cochlea with three aspirations of neomycin. b) Response from (a) plotted against the stimulus intensity.
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Figure 6.14: a) ABR recording from electrical stimulation alone and b) hybrid electrical-optical stimulation. INS laser with fibre aimed at Rosenthal’s canal inside the chronically deafened cochlea.

may have been due to electrode movement during the experiment. An example of the response is shown in Figure 6.14. At 275 $\mu$A a small response can be seen for the hybrid stimulation, while no response is apparent for the electrical only case until 300 $\mu$A. This is a smaller absolute reduction in threshold than that observed for the acutely deafened animal, but the relative improvement is similar a similar range of 8 – 9%.

Overall, in profoundly deaf animals, hybrid electrical-optical stimulation displayed a reduced threshold compared to electrical stimulation alone. The mechanism behind this reduction is not clear. Infrared stimulation was unable to evoke a response in isolation even with radiant exposures known to activate peripheral nerves such as the sciatic nerve. This suggests that the usual mechanisms behind INS may not have been active here. One possibility is that the laser heating increases the sensitivity of the nerves to stimuli (Collins and Rojas, 1982). While infrared stimulation is active, the baseline temperature of the tissue will increase (see Chapter 4), which could then account for the observed reduction in thresholds.

6.4 Possible mechanisms of infrared stimulation in the cochlea

Overall, no clear response to infrared stimulation was observed in the eight profoundly deaf guinea pig cochleae using four different laser sources. A modest reduction in threshold could be observed when combined with electrical stimulation, but
6.4. Possible mechanisms of infrared stimulation in the cochlea

Figure 6.15: Figure 4 from (Teudt et al., 2011). Hydrophone signal in mPa from 100 µs duration pulse (E = 110 µJ). Figure from (Teudt et al., 2011), is copyright IEEE and is used with permission.

Further work is required to ascertain how strong this effect is and to ascertain the mechanisms behind it. This section explores the potential involvement of hair cells in the mechanism of infrared stimulation of the cochlea, rather than a direct interaction as previously suggested. Two potential mechanisms for infrared stimulation will be discussed: a response due to the acoustic click generated by the lasers used and a direct interaction between hair cells and the infrared laser.

Although the laser pulses used for INS are significantly longer than stress confinement (Wells et al., 2007a; Richter et al., 2011a), it has been shown that they can create acoustic events in water (Fig 6.16) or a humid atmosphere (Teudt et al., 2011) (Fig 6.15 and Fig 5.19). The cochlea is an organ specialised to detect sound and has previously been shown to detect these acoustic events (Teudt et al., 2011). This optoacoustic process is therefore the most obvious explanation for the results shown in this thesis and in previous reports of INS in the cochlea. Additionally, a number of papers have demonstrated the use of laser-induced optoacoustic sounds to stimulate the inner hair cells (Schultz et al., 2012; Wenzel et al., 2009; Zhang et al., 2009).

However, as discussed below, a purely optoacoustic mechanism is not consistent with all of the reported results. An alternative to an optoacoustic mechanism is an interaction between the infrared laser and the hair cells of the cochlea. As hair cells show a response to infrared light (Rajguru et al., 2011; Liu et al., 2013), it is possible that this form of excitation may play a role in some of the responses seen.

It could be argued that the total number of animals used for this thesis (n = 8) and with the Aculight Renoir laser (n = 4) is too low to make strong statements about the basis of INS. However, without a clear avenue to advancing the procedure for further experiments and an expectation that this would change the outcome,
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6.4.1 Optoacoustic Mechanism

Photomechanical and optoacoustic effects have generally not been considered in the INS literature, as the pulse lengths used are much longer than stress confinement (Richter et al., 2011a). In the context of laser-tissue interactions, stress confinement describes the time required for a stress wave to traverse the heated volume (Welch and van Gemert, 2011). For a one-dimensional case it can be defined as $t_m = \frac{1}{\mu_a c_a}$, where $c_a$ is the speed of sound in the medium. For the measurements shown in Fig 5.19 and those reported by Teudt et al. (2011), the small fibre diameter is more restrictive to the size of the heated volume. Therefore using the radius of the heated region is a better approximation of the stress confinement time $t_m = r_{\text{fibre}} / c_a$. For a $\phi_{\text{fibre}} = 200 \, \mu m$ in water ($c_a = 1497 \, \text{m.s}^{-1}$ (Welch and van Gemert, 2011)), a stress confinement time of 67 ns is found. In this regime, the pressure can be found by $\delta p = \Delta T \beta B$, where $\beta = 2.97 \times 10^{-6} \, ^\circ\text{C}^{-1}$ is the coefficient of thermal expansion and $B = 2.2 \times 10^9 \, \text{Pa}$ is the bulk modulus (Welch and van Gemert, 2011; Paltauf and Dyer, 2003). From this, the pressure can be estimated from the stress confined component of microsecond pulse by using the model of Part I to calculate the temperature change. Teudt et al. (2011) found a 30 mPa pressure change from a 100 $\mu s$ duration pulse with 110 $\mu J$ energy (Fig 6.16). The temperature change from the first 67 ns would be 0.33 m$^\circ$C. In water, this temperature change would result in a pressure increase of $\delta \rho = 153 \, \text{Pa}$, well above the 30 mPa reported by Teudt et al. (2011).
Much of this discrepancy can be explained by a reduction of pressure in the 5 mm between the fibre and hydrophone, as sound is attenuated according to $\propto 1/r$. Taking this distance into account, a pressure increase of $\delta \rho = \frac{100 \, \mu{\text{m}}}{5 \, \text{mm}} \times 153 = 3 \, \text{Pa}$ is found, still two orders of magnitude greater than reported by Teudt et al. (2011). This basic calculation of pressure gives results much greater than those reported by experimental measurements, which suggests that a weak stress confinement component provides a plausible explanation of sound generation for microsecond laser pulses. In addition to directly stimulating hair cells, this pressure wave may also induce vibrations in the basilar membrane (Ren et al., 2014).

Although a purely optoacoustic explanation seems plausible, as much of the cochlea INS literature is in normal hearing animals (Izzo et al., 2007c,b, 2008b; Littlefield et al., 2010; Matic et al., 2011; Goyal et al., 2012; Matic et al., 2013), it is inconsistent with some of the literature on INS where partial deafening has been performed (Izzo et al., 2006; Richter et al., 2008; Moreno et al., 2011; Richter et al., 2011b). For example, Figure 6.17 (reproduced from Izzo et al. (2006)) shows a reduction in optical CAP amplitude up to 40 minutes after deafening gerbils with kanamycin and ethacrynic acid injections, while auditory CAP thresholds increase by $\sim 60 \, \text{dB}$ in the same time. This deafening technique has been shown to profoundly deafen the cochlea within the order of 20 – 30 minutes (Xu et al., 1993). Use of kanamycin and ethacrynic acid in cats has been shown to produce a more profound deafness in the basal, or high frequency, region of the cochlea while leaving less damage more apically (Shepherd and Martin, 1995). Therefore, as an alternative explanation to INS, it is possible that the 4 kHz acoustic tone was in the region more affected by the treatment, while the infrared laser illumination was acting more apically. However, without further evidence this suggestion is not very robust. Additionally, animals chronically deafened with neomycin still responded to infrared laser illumination, albeit at greatly increased radiant exposures, and histological examination of the cochlea found no remaining hair cells (Richter et al., 2008).

A similar discussion can be had about the results presented in (Richter et al., 2008). In this paper, thresholds for INS were investigated in non-deafened control, acutely deafened and chronically deafened animals. Figure 6.18 shows the change in sound levels required to evoke a 30 $\mu$V CAP in chronically deafened gerbils, four weeks after application of neomycin. Four weeks before the experiment, gerbils were deafened using a trans-tympanic injection of 100 – 150 $\mu$L of Ringer’s Lactate containing neomycin at varying concentrations of 5, 10, 25, 50, 75 or 100 mM. Neomycin concentrations were different in each ear and grouped as follows: 50/100 mM (right/left ear) in six animals, 25/75 mM in seven animals and 5/10 mM in six
Figure 6.17: Figure 2a from (Izzo et al., 2006), showing the change in response as an animal is acutely deafened. A large increase in the acoustic CAP threshold (solid squares) at 4 kHz is observed, while only a small reduction in oCAP threshold (open squares) is seen. Figure from (Izzo et al., 2006), is copyright John Wiley and Sons and is used with permission.

Figure 6.18: Figure 2 from (Richter et al., 2008), showing the average sound levels required to evoke a 30 µV CAP for control animals and deafened with neomycin four weeks before assessing hearing thresholds. No response could be evoked for neomycin concentrations of 25 mM or greater, therefore the filled circles show the maximum sound level of the system. Figure from (Richter et al., 2008), is copyright Elsevier and is used with permission.
animals. For a neomycin concentration of 10 mM, only a ~15 dB increase in acoustic thresholds is observed, while for the 25 mM and greater concentrations, the threshold was at or beyond the maximum level of the speaker and no response could be evoked. No animals with 50 mM or greater concentrations of neomycin responded to any stimulus, whether electrical, acoustic or optical. Additionally, not all of the animals with 25 mM concentrations responded to the various stimuli. Histological analysis of the cochleae after the experiments revealed a significant reduction in spiral ganglion cell counts for increased concentrations of neomycin. For example at the base of the cochlea, the control SGN cell density was $1010 \pm 113$ cells mm$^{-2}$, $1070 \pm 348$ cells mm$^{-2}$ at 10 mM neomycin, $541 \pm 453$ cells mm$^{-2}$ at 25 mM neomycin, $369 \pm 96$ cells mm$^{-2}$ at 50 mM neomycin, $377 \pm 18$ cells mm$^{-2}$ and $368 \pm 71$ cells mm$^{-2}$ at 100 mM neomycin.

The average radiant exposures that were required to evoke an optical response in (Richter et al., 2008) are shown in Figure 6.19. The results for the chronically deafened animals include those treated with 10 mM and 25 mM of neomycin that also responded to electrical stimulation. However, the standard deviation for many of the values is of the order, if not greater, than the mean, implying a large spread in thresholds between the different cochleae tested. An alternative interpretation of this data is that some cochleae responded at a similar threshold to the acute and control animals, while some required a significantly increased radiant exposure due to greater hair cell loss. This speculation is strengthened by the 80 – 90% relative standard deviation for SGN density with the 25 mM concentration of neomycin, which suggests variable deafening between the different animals. Therefore this result would be consistent with cochleae that exhibited residual hearing responding to laser stimulation, while those that were profoundly deafened did not.

Moreno et al. (2011) examined the target structures of INS using a combination of inferior colliculus recordings, histological reconstructions, microCT scans, and marking with CO$_2$ laser ablation. Neomycin was added to the scala tympani, before experimentation with INS but after measuring acoustic thresholds and finding the placement of the inferior colliculus electrode. After addition of neomycin no CAPs could be evoked in response to frequencies of 15 kHz or above and CAP thresholds were elevated by at least 40 dB in the rest of the hearing range. The cochlea was then stimulated using an Aculight Capella laser at a wavelength of 1862 nm, pulse length of 100 $\mu$s and pulse energies up to 127 $\mu$J. Recordings were made in the inferior colliculus to assess the spatial localisation of stimulation. After INS experimentation was complete, the optical fibre was removed and replaced by a hollow fibre in the same orientation to ablate tissue using 10.6 $\mu$m wavelength infrared light from a
Figure 6.19: Table 1 from (Richter et al., 2008), is copyright Elsevier and is used with permission.

Table 1

<table>
<thead>
<tr>
<th>Pulse length (µs)</th>
<th>Control (J/cm²)</th>
<th>Acutely deaf (J/cm²)</th>
<th>Chronically deaf (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.003 ± 0.001</td>
<td>0.006 ± 0.003</td>
<td>0.012 ± 0.002</td>
</tr>
<tr>
<td>(N = 10)</td>
<td>(N = 9)</td>
<td>(N = 3)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.006 ± 0.003</td>
<td>0.007 ± 0.002</td>
<td>0.065 ± 0.091</td>
</tr>
<tr>
<td>(N = 0)</td>
<td>(N = 10)</td>
<td>(N = 3)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.007 ± 0.010</td>
<td>0.009 ± 0.002</td>
<td>0.164 ± 0.312</td>
</tr>
<tr>
<td>(N = 11)</td>
<td>(N = 10)</td>
<td>(N = 4)</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.014 ± 0.006</td>
<td>0.014 ± 0.006</td>
<td>0.272 ± 0.523</td>
</tr>
<tr>
<td>(N = 10)</td>
<td>(N = 10)</td>
<td>(N = 6)</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>0.012 ± 0.010</td>
<td>0.017 ± 0.001</td>
<td>0.559 ± 0.104</td>
</tr>
<tr>
<td>(N = 10)</td>
<td>(N = 10)</td>
<td>(N = 6)</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>0.020 ± 0.087</td>
<td>0.020 ± 0.040</td>
<td>0.719 ± 1.262</td>
</tr>
<tr>
<td>(N = 10)</td>
<td>(N = 9)</td>
<td>(N = 6)</td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>0.020 ± 0.060</td>
<td>0.037 ± 0.030</td>
<td>0.887 ± 0.107</td>
</tr>
<tr>
<td>(N = 0)</td>
<td>(N = 10)</td>
<td>(N = 7)</td>
<td></td>
</tr>
</tbody>
</table>

The averages for the chronically deaf animals include animals that were deafened with neomycin concentrations of 10 and 25 mM and only include the animals for which a CAP could be evoked with an optical radiation pulse. For neomycin concentrations of 50 mM and larger, radiant exposures up to 127 mJ/cm² or electric currents pulses up to 1 mA did not evoke a measurable CAP or ABR.

CO₂ laser.

Figures 6.20 and 6.21 show 3D reconstructions and spatial tuning curves adapted from Figures 4 & 5 of (Moreno et al., 2011). The 3D reconstructions are derived from histological reconstructions and ablation from a CO₂ laser. They show the centre of the spiral ganglion neurons (black), inner pillar feet (support cells of the inner hair cells, green), cochleostomy (blue), ablated sites (red) and the position of the optical fibre (black cylinder). Figure 6.20b suggests that there are two areas of excitation, one at 10.8 kHz and another at 16.1 kHz. Figure 6.21b shows excitation at 4 kHz, which is more apical than expected based upon the cochleostomy. Other results shown by Moreno et al. (2011), but not reproduced here, show results where broad stimulation occurred over a range of sites. These results suggest that stimulation is possible in turns of the cochlea on the opposite side of the fibre, in addition to directly near the optical fibre targeting the neurons. The authors state that the auditory nerve in the centre of the modiolus was not in the beam path and that stimulation of the auditory nerve is unlikely to explain the broader response seen in some cases. Additionally, the 3D reconstructions suggest that the inner hair cells in more apical turns may be exposed to infrared pulses.

A further argument for hair cells being the primary target of infrared stimulation comes from the wavelength dependence of CAP amplitudes presented by Izzo et al. (2007c). The laser used for this study could have its wavelength varied over the range 1844 – 1873 nm. This wavelength range corresponds to a water absorption range of $0.91 \leq \mu_a \leq 2.3$ and penetration depths of $1100 \mu m > z_a > 435 \mu m$. At the longest wavelength, corresponding to the shortest penetration depth, CAP threshold
6.4. Possible mechanisms of infrared stimulation in the cochlea

Figure 6.20: A. 3-D reconstruction of a guinea pig cochlea, where green indicates inner pillar feet, black dots are the centre of the spiral ganglion neurons, blue is the cochleostomy, red is the centre of the site that was ablated and the black cylinder shows the optical fibre position. B. shows the spatial tuning curve obtained from recordings of the ICC, showing stimulation at 10.8 kHz and 16.1 kHz. Figure is adapted from Figure 4 of (Moreno et al., 2011), which shows additional rotations. Figure from (Moreno et al., 2011), is copyright Elsevier and is used with permission.

Figure 6.21: A. 3-D reconstruction of a guinea pig cochlea (see Fig. 6.20 for details) B. shows the spatial tuning curve obtained from recordings of the ICC, showing stimulation primarily at 4 kHz. Figure is adapted from Figure 5 of (Moreno et al., 2011), which shows additional rotations. Figure from (Moreno et al., 2011), is copyright Elsevier and is used with permission.
was found. Keeping the same radiant exposure, the wavelength was adjusted to shorter wavelengths. The results shown in Figure 6.22b show a rapid increase in the CAP amplitude from a wavelength of 1873 nm to $\sim 1860$ nm, where the increase in response reaches a plateau. The authors state that the targeted spiral ganglion neurons were approximately 500 $\mu$m from the optical fibre (as shown in Figure 6.22a). However, both the Monte Carlo model (Figure 6.23) and a simple analytical examination of heating show that the temperature increase 500 $\mu$m from an emitter is greater at 1873 nm. At distances of 750 $\mu$m and greater, the temperature increase is greater in less strongly absorbed wavelengths. If the mechanism of INS in the cochlea is due to water absorption, this suggests that the target of stimulation is beyond the spiral ganglion neurons in the upper basal turn. Alternatively, there may be a chromophore present in the target, making the process dependent on radiant exposure rather than temperature (as previously discussed in Section 2.4.3).

6.4.2 Other mechanisms targeting hair cells

An optoacoustic mechanism is not the only possible explanation for the lack of response to infrared stimulation observed in profoundly deaf animals. This section presents a number of possible alternatives, including alternative interactions between infrared radiation and hair cells, and the changes that deafening makes to the neurological behaviour of the cochlea.
Vestibular hair cells have been shown to respond to infrared stimulation (Rajguru et al., 2011). There is evidence that this was not an optoacoustic response, as increasing infrared intensity did not reduce the latency to stimulation (Rajguru et al., 2011). Furthermore, it has been shown that infrared laser pulses evoked miniature post-synaptic currents in the hair cells (Liu et al., 2013). However, as the INS lasers are typically not directed towards the hair cells and scattering is low at the wavelengths used for INS, meaning little light will be incident on the hair cells, this explanation seems relatively unlikely for hair cells near the fibre. However, the results shown by Moreno et al. (2011), suggested excitation on the side of the cochlea opposite to the optical fibre. Therefore, residual hair cell activity, could be involved with infrared stimulation of the cochlea.

It has previously been shown that neomycin may act as a calcium channel blocker (Zhou et al., 1996; Canzoniero et al., 1993). Indeed, blocking the outer hair cell ACh receptor channel (Blanchet et al., 2000) and blocking of Ca\(^{2+}\) activated K\(^{+}\) channels (Dulon et al., 1995) have been shown as a mechanism of immediate aminoglycoside deafening, as previously discussed in Section 1.2.1. As calcium is involved with INS (Dittami et al., 2011), blocking of calcium channels may interfere with the mechanisms of infrared stimulation. It has been proposed that the greater quantity of neomycin used for the deafening protocol in this thesis\(^5\), compared to that used in references such as (Richter et al., 2008), may act as a channel blocker and thus

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\(^5\)The protocol used in this study is a standard procedure of the Bionics Institute
prevent the nerves from responding (Richter, 2013). This hypothesis was tested with the following techniques: damaging the middle ear to cause conductive hearing loss to examine the response with reduced hearing function; and infrared stimulation in chronically deafened animals, as any ototoxic aminoglycosides should no longer be present in the cochlea, thus reversing any blocking of ion channels or transduction channels (Lim, 1986).

In this work, the two control and acutely deafened animals showed different responses to infrared stimulation. As described in Section 6.2.1, in surgery on the second animal examined, a larger exposure of the cochlea was performed, resulting in damage to the middle ear. This loss of conductive hearing caused an increase on the order of 35 dB in the response threshold to an acoustic click stimulus. Unlike the first animal, no response could be evoked from the infrared laser alone before deafening, regardless of whether the emitter was next to the cochlea or inside and directed towards the spiral ganglion neurons. This result suggests that the response observed due to infrared stimulation may be partially due to conduction or amplification by middle ear of the acoustic click generated by the laser. However, the trauma from exposing the cochlea and performing a cochleostomy can cause hearing loss due to hair cell death in response to the severe vibrations and acoustic stimulus (Bas et al., 2012). As the surgery on the second animal was more severe than the first, it is possible that this killed or deactivated the hair cells and was responsible for the lack of response seen, rather than it being due to the middle ear damage.

When examining infrared stimulation in chronically deafened animals, no clear response to infrared stimulation of any pulse lengths was observed in the four cochleae tested, as described in Section 6.2.2. Additionally, cochleostomies were performed in more apical turns in two cochleae as there is often a higher rate of SGN survival in this position after chronic deafening (Leake and Hradek, 1988). Despite this, no response was observed. As there is a high rate of SGN degradation in chronically deafened animals and the amplitude of the stimulation response depends on the number of neurons excited (Richter et al., 2008; Hardie and Shepherd, 1999; Hall, 1990) it is potentially possible that this degradation would hide the optical response and that neomycin could hinder the response in acutely deafened animals. In vitro experimentation may assist in revealing the role of neomycin in acutely deafened animals and how it influences the response in acute preparations.

Thresholds used for INS in the cochlea have typically been lower than that for INS in other targets (as summarised in Fig 1.9). One explanation for this discrepancy has been that spontaneous neural activity in the cochlea provides a baseline level and INS activates an additional number of neurons which results in a recordable ABR or
6.4. **Possible mechanisms of infrared stimulation in the cochlea**

CAP (Richter, 2013). After deafening the cochlea, this spontaneous activity reduces or disappears (Schmiedt et al., 1980; Shepherd and Javel, 1997; Matsushima et al., 1991; Hartmann and Klinke, 1989). Therefore, the loss of spontaneous activity in the neurons caused by the death of hair cells may also prevent INS from functioning. As a result, it is possible that INS is not optoacoustic, but still depends on a residual level of hearing or typical cochlea function. This would limit the potential for INS as a neural stimulation technique in an implantable device.

Recent work by Okunade and Santos-Sacchi (2013) has shown that the motor protein in outer hair cells, prestin, can respond to rapid temperature changes such as those produced by exposure to infrared illumination. Prestin drives the rapid mechanical changes of the outer hair cells (Zheng et al., 2000), which allows these cells to amplify vibrations in the cochlea. Prestin knockout mice have shown an increase in acoustic thresholds on the order of 35 – 60 dB (Cheatham et al., 2004), highlighting the critical role of prestin in amplifying small acoustic vibrations of the basilar membrane. Prestin is known to rapidly respond to many energy forms including mechanical (Frank et al., 1999) and electrical (Kachar et al., 1986). These rapid transitions allow the outer hair cells to respond at acoustic frequencies up to the mammalian limit of roughly 100 kHz (Ashmore, 2008).

Okunade and Santos-Sacchi (2013) transfected human embryonic kidney (HEK) cells with prestin (SLC26a5) and exposed them to infrared laser radiation under whole-cell voltage clamp. An Aculight Capella R-1850 laser with a maximum power of 5 W was delivered via a 600 µm core diameter fibre that was positioned within 500 µm of the target cells. Pulse durations of 20 ms at 40% power (n = 5) or 5 ms at 95% power (n = 2) were used. No measurements of the pulse energy or laser power were given and it was unclear whether the relationship between percent laser and actual laser power emitted is linear. Compared to control HEK cells, which showed a monophasic increase in membrane capacitance, the transfected cells displayed nonlinear capacitance, with a biphasic change in membrane capacitance depending on voltage. This nonlinear capacitance is characteristic of prestin and indicates that it responds to changes in temperature at a similar speed as the mechanical or electrical response. This could allow the outer hair cell behaviour to be modified by infrared heating.

Given this response observed in prestin due to infrared heating, if outer hair cells are exposed to infrared laser light, it is possible that this could mediate the response seen during infrared stimulation and it could potentially also enhance the detection of optoacoustic signals by inner hair cells. In response to activation of the outer hair cells, a cochlear microphonic (CM) is usually seen in CAP recordings (Patuzzi et al.,
Chapter 6. Exploring Infrared Stimulation of the Cochlea

1989; Dallos and Cheatham, 1976; Dallos and Wang, 1974). Izzo et al. (2006) noted that no cochlea microphonic was observed in CAP recordings with laser stimulation, while acoustic stimuli generated a cochlea microphonic response. Although it is possible that infrared activation of the outer hair cells does not generate cochlear microphonic, it is less likely that they are activated during INS of the spiral ganglion neurons. Additionally, it is worth noting that the temperature changes induced by Okunade and Santos-Sacchi (2013) were on the order of 10 °C and are much greater than the changes typically involved with INS in the cochlea, which are on the order of 0.1 °C. Given the two orders of magnitude difference in temperature change and pulse length, and lack of cochlear microphonic in the CAP response to INS, it is unclear whether the response of prestin to IR heating shown by Okunade and Santos-Sacchi (2013) is involved with INS in the cochlea. Unravelling the exact interactions of infrared laser stimulation with different components of the auditory system will require further experimentation.

The 3D reconstruction of the cochlea and laser beam path during INS by Moreno et al. (2011) showed responses from more apical turns of the cochlea, where the organ of Corti and hair cells are exposed to infrared light in addition to the spiral ganglion neurons (Figs 6.20 & 6.21). Although neomycin was added to deafen the cochlea, a threshold elevation of only 40 dB for frequencies below 8 kHz (Moreno et al., 2011) was applied, rather than ensuring the cochlea to be profoundly deaf. Given that outer hair cells are more susceptible to aminoglycoside-induced damage compared to inner hair cells (Sha et al., 2001; Dallos and Harris, 1978; Ding et al., 2010; Lim, 1986) and that the loss of outer hair cell function causes 40 – 60 dB of hearing loss (Cheatham et al., 2004), it is possible that a significant population of functional inner hair cells remained and mediated the response observed. This suggestion is strengthened by one example (Fig 6.21) showing the absence of a response near the fibre but a strong response on the far side of the cochlea. The results presented by Moreno et al. (2011) are consistent with inner hair cells mediating the response, as well as a direct interaction with spiral ganglion neurons, as argued by the authors.

In comparing the results from this thesis with previously published results and in searching for an understanding of the mechanisms and target structures of infrared stimulation, the results from the initial lasers can be discounted. Only the Aculight laser showed a clear response in a normal hearing animal, despite the other lasers providing greater radiant exposure than the previously published works. Laser MK I was only tested in a profoundly deaf animal and therefore, no clear response would be expected if hair cells are involved with the response of infrared stimulation. Laser MK II was only marginally above threshold, although larger core diameters have
shown a lower threshold for INS of the sciatic nerve (Table 1.1). Laser MK III was clearly above thresholds from the literature and it is unknown why no response was observed. Compared to the Aculight laser, only a hint of an acoustic click could be recorded smaller than expected for the change in laser power. It is unclear what makes this laser less efficient at generating an acoustic response.

With the Aculight Renoir laser, the responses between the two control/acute deafened animals were distinctly different. The first animal responded to both optoacoustic stimulation, with the fibre positioned in the bulla, next to but not pointing at the cochlea (Fig 6.6); and to stimulation with the fibre inserted through a cochleostomy inside the cochlea and directed towards the spiral ganglion neurons (Fig 6.8). After deafening the cochlea with repeated aspirations of neomycin through the scala tympani, no response could be evoked.

Compared to the first animal, the second animal used for the control/acute deafening experiment responded very differently to infrared stimulation. This difference may have been due to middle ear damage, causing an acoustic threshold elevation of approximately 35 dB. No response from an optoacoustic mechanism could be observed when the fibre was positioned in the bulla, next to but not directed towards the cochlea. With the fibre positioned in the cochlea and directed towards the spiral ganglion neurons, no response could be evoked from infrared stimulation. If absence of a response is ascribed to the conductive hearing loss, it suggests that infrared stimulation is mediated by hair cells and amplification in the middle ear. However, this does not agree with the observation by Moreno et al. (2011) that the exposure of nerves or hair cells due to the fibre position correlates to resultant spatial tuning curve (see Figs 6.20 and 6.21). A purely optoacoustic explanation for infrared stimulation would not explain the spatial localisation observed by Moreno et al. (2011). Another explanation for this discrepancy between experiments is that the more severe surgery may have damaged the hair cells by over exposing them to excessive vibration and noise (Bas et al., 2012), preventing any clear response from being observed.

The results from the two chronically deafened animals showed no clear response to infrared stimulation, despite exposing apical turns of the cochlea to INS where spiral ganglion neuron degeneration is generally less severe. These experiments reduced the plausibility of aminoglycosides acting as a channel blocker on spiral ganglion neurons, as the drugs should have left the system after four weeks (Aran et al., 1999; Smith et al., 1994). Richter et al. (2008) observed a correlation between increasing dose of neomycin and reduced probability of response to INS. The authors argued that this reduction was due to the reduced population of spiral ganglion neurons and additionally they saw no response from electrical stimulation where there was
no optical response. The results in this thesis from chronically deafened animal experiments are more consistent with infrared stimulation being mediated by hair cells, as no optical response was observed despite the cochleae giving typical electrical responses.

Overall, the primary difference between the study presented here and those of the published literature is the deafening techniques used. The majority of the INS cochlea studies are in normal hearing animals (Izzo et al., 2007c,b, 2008b; Littlefield et al., 2010; Matic et al., 2011; Goyal et al., 2012; Matic et al., 2013). In those with deafening, a residual level of hearing remained. The deafening process reported by Izzo et al. (2006) increased acoustic thresholds by 30 – 60 dB at 4kHz. Applying neomycin to the intact round window increased thresholds by 40 – 60 dB in acutely deafened animals (Richter et al., 2008), while only 20 – 30 dB increases in threshold were observed for chronically deaf cochleae that responded to INS. Moreno et al. (2011) and Richter et al. (2011b) injected neomycin solution into the cochlea, but did not aspirate it through. Animals used by Moreno et al. (2011) did not respond to acoustic stimuli above 15 kHz and showed threshold increases of at least 40 dB for frequencies below 8 kHz. Finally, Richter et al. (2011b) reported no response for acoustic stimuli with frequencies above 20 kHz, but a 20 dB threshold elevation at 8 kHz.

In the present work, acute deafening was performed with multiple aspirations of neomycin through the cochlea. Compared to the deafening technique used in much of the work published by the Northwestern group (e.g. (Richter et al., 2008; Moreno et al., 2011)), this technique ensures a more thorough exposure of the hair cells to neomycin and is well established to leave the cochlea profoundly deaf (Hardie and Shepherd, 1999). It is important to thoroughly expose the cochlea to neomycin as apical hair cells are typically less susceptible to neomycin (Sha et al., 2001; Dallos and Harris, 1978; Ding et al., 2010). Therefore, simply allowing neomycin to perfuse through the cochlea (Moreno et al., 2011; Richter et al., 2011b) may not ensure adequate exposure at the apex which could leave the cochlea with significant hearing and would provide a mechanism for detection of the acoustic events generated by the laser absorption in water. Similarly, where a response has been observed in chronically deafened animals in the literature (Richter et al., 2008), it can be argued that the deafening was insufficient as an optical response was only observed when an acoustic response could also be detected in the same cochlea. This is an important distinction, as the target patients for cochlear implants typically have severe to profound deafness with few if any remaining hair cells. For INS to be viable in a cochlear implant, it needs to be demonstrated in cochleae without functioning hair
Hybrid electrical-optical stimulation showed the potential to reduce electrical stimulation thresholds in profoundly deaf cochleae by 25 – 50 µA, a reduction on the order of 8 – 10%. At this stage the process is not optimised and further reductions could be expected. Since the initial reports of hybrid stimulation by Duke et al. (2009) the process was improved, increasing the reliability and reducing optical thresholds by up to 90% (Duke et al., 2012a). The mechanism behind this reduction is not clear, although the most likely candidate is an increase in neuron excitability due to heating (Collins and Rojas, 1982). These results point to an interaction between the laser and neurons in the cochlea. However, uncertainty of the mechanism behind the electrical threshold reduction makes it difficult to use this observation to understand the mechanism of infrared stimulation alone. Additionally, any increase in spatial localisation of stimulation is, at this stage, unknown. With significant optimisation of the process, hybrid electrical-optical stimulation may present opportunities for clinical use in cochlear implants.

In summary, it is plausible that the response to infrared stimulation in the cochlea is a combination of two effects mediated by residual activity in the hair cells: an optoacoustic response to the laser pulse and a direct interaction between the infrared light and hair cells. The optoacoustic response has been shown in this thesis and previously by Teudt et al. (2011) with the optical fibre positioned next to, but not directed towards the cochlea. However, alone this mechanism does not support the spatial localisation of stimulation to the beam path shown by Moreno et al. (2011) and it is unclear how moderate chemical deafening, leaving some residual hearing, corresponds to the response still observed. Therefore, a direction interaction between the infrared light and hair cells may be postulated to explain this phenomenon. A number of possible candidates exist to explain this interaction, including: prestin in the outer hair cells responding to heating from infrared irradiation (Okunade and Santos-Sacchi, 2013); an interaction between the hair cells and infrared radiation, which has previously been shown in the vestibular system (Rajguru et al., 2011); and post-synaptic currents evoked by exposure to IR lights in inner hair cells (Liu et al., 2013). It is unclear how hair cells respond to moderate chemical deafening, but given that inner hair cells are less susceptible to aminoglycosides than outer hair cells (Dallos and Harris, 1978; Sha et al., 2001; Ding et al., 2010), an infrared light modulation of residual function may explain results that can’t be attributed to an optoacoustic effect. However, at this point, any discussion of potential mechanisms remains speculative. Identifying the exact mechanisms behind the reported results of infrared stimulation in the cochlea may require further in vivo work and in vitro
studies in tissue slices and cultured auditory neurons.

6.5 Conclusion

Four different laser sources were attempted for infrared stimulation in the cochlea. All except the MK I laser were used in both normal hearing and profoundly deaf animals, but only the Aculight laser was able to elicit a response and that response was only observed in a normal hearing animal. Consistently negative results were observed in profoundly deafened animals, despite the other lasers having a radiant exposure greater than the previously published thresholds. Through use of histological analysis, the optical fibre coupled to the laser was confirmed to be correctly targeting the spiral ganglion neurons. These results suggest that the target of infrared stimulation may not be spiral ganglion neurons, as has generally been suggested, and that some residual hair cell activity may have been involved in previously reported results. Overall, an optoacoustic mechanism or other involvement of functional hair cells appears to be consistent with the experimental results presented in this Chapter, as no response could be evoked in a profoundly deaf cochlea.

It should be noted that the result presented here do not provide any insights into the mechanisms of INS in other neural targets. This is an important distinction, because typical thresholds seen in previous reports of stimulation in the cochlea with INS are 1 – 2 orders of magnitude below those seen for other neural targets (Fig 1.9). However, while it can also be argued that hair cell involvement is consistent with many of the results in the published literature, no definitive statements can be made in this regard. This does not rule out the influence of the other speculative mechanisms discussed here, including a contribution from infrared exposure of outer hair cells assisting in amplifying the acoustic stimulus. Without a clear response in profoundly deaf cochleae, the benefit of INS in the cochlea and its potential for therapeutic use in a bionic device is questionable, as many of the target patients will have both spiral ganglion degradation and a lack of functioning hair cells.
7.1 Thesis Summary

This thesis presents studies undertaken to develop a detailed model of heating during INS (Part I), taking into account spatial and temporal effects to compare different fibre sources, wavelengths, pulse durations and stimulation targets. Results from \textit{in vivo} experimentation with infrared stimulation in guinea pig cochleae, using a number of different lasers sources and deafening techniques are presented in Part II. These results are compared with the literature in an attempt to understand the mechanism behind reports of INS in the cochlea. In this final chapter, the main conclusions from this work are summarised and some suggestions for future work are discussed.

7.1.1 Modelling

The thermal model of INS introduced in Part I provides detailed information on both the spatial (Chapter 2) and temporal distribution (Chapters 3 and 4) of heat during INS. It predicts that for published results of INS in the cochlea, the temperature increase from a single pulse is typically on the order of 0.1 °C when using a pulse energy of 25 µJ and the most commonly used wavelength of $\lambda = 1850$ nm. With lower pulse energies, down to 2.5 µJ, the temperature increase can be as low as 0.01 °C.
When the model is applied to the stimulation of peripheral nerves, such as the sciatic nerve, or in vitro studies, the predicted changes in temperature are much higher than those found in the cochlea, on the order of 1 °C to 10 °C. The predictions of the model for in vitro and in vivo geometries agree well with experimental measurements.

The model of the spatial behaviour of heating, presented in Chapter 2, shows that heat is localised to the region directly exposed to the laser irradiation. This supports experimental reports of strong localisation compared to electrical stimulation and shows promise for application development. Additionally, it shows how spatial localisation in the z-axis can be achieved with a combination of fibre properties and wavelength optimisation.

The temporal model of heating, as presented in Chapters 3 and 4, provides details of heat flow during and after INS pulses. A more detailed understanding of the heat conduction was found to be especially important during longer pulses of INS, where heat flow has a larger effect. Both the spatial and temporal aspects of the model show where previously used “rules of thumb” are valid and where a more detailed approach is required. Importantly, when comparing the temporal behaviour in regions of different size, such as those due to different fibre core diameters, the model shows that smaller regions of excitation have a faster thermal decay. Rapid thermal decay potentially allows for higher pulse repetition rates without causing dangerous heating, and the higher pulse rates are critical for delivering sufficient information in some neural prostheses. When applying the model that has been developed to repetitive pulses, it allows for comparisons between different fibre core diameters and pulse rates and the results are able to provide a guideline when comparing between different stimulation rates. Furthermore, the model suggests that the use of multiple emitters with tighter spacing is feasible in cochlear devices without the risk of cross talk or a greatly increased thermal load.

When results of the model were compared to experimental measurements by Shapiro et al. (2012), the predictions of both maximum temperature achieved and thermal decay rate correlated well with the observations.

### 7.1.2 Infrared stimulation in the cochlea

The experimental results presented in Part II of this thesis suggest that the target of INS in the cochlea may be hair cells, rather than spiral ganglion neurons as generally suggested. A range of different laser sources were attempted for INS (Chapter 5) in normal hearing and profoundly deaf animals (Chapter 6). No response was observed to the optical stimulus in any profoundly deaf animal, this implies either that normal hearing function or some residual hair cell activity is required for the cochlea to
7.1. Thesis Summary

respond to INS. Nor was any response evoked with lasers other than the Aculight source, despite radiant exposures for these lasers being above previously published thresholds.

The results presented in Chapter 6 in combination with analysis of previously published results suggest there may be two separate mechanisms behind the hair cell mediated response. Previous work by Teudt et al. (2011) showed a clear optoacoustic response in the cochlea, which the authors argued may contribute to reports of INS in the literature. In this work a clear optoacoustic response from INS in the cochlea was also observed when the fibre was positioned outside the cochlea and not directed towards neurons or hair cells. When the fibre was aimed towards the spiral ganglion neurons, a response was evoked in a normal hearing animal but not in any profoundly deaf animals. This suggests that normal functioning hair cells are required to evoke a response, whereas there may have been incomplete deafening in some previous work (see below). An optoacoustic mechanism is additionally supported by the absence of any response in an animal with conductive hearing loss which occurred due to damage of the middle ear.

It is unclear how the optoacoustic mechanism applies to previously reported results where chemical deafening reduced acoustic thresholds by $20 - 40$ dB (e.g. Richter et al. (2008)), compared to $>50$ dB used in this study. Impairment of hearing function by $20 - 40$ dB would be expected to prevent any response from the laser-generated click; however, a response was still present without a large shift in threshold (Richter et al., 2008). Additionally, results by other researchers showed a good correlation between the position of an optical fibre used for excitation and hair cells on the far side of the cochlea (Moreno et al., 2011). Therefore, it is plausible that an alternative to the optoacoustic mechanism exists in some cases of INS in the cochlea and that the two effects may combine in a normal hearing animal when hair cells are in the beam path. A number of possible candidates exist to explain this, including post synaptic currents evoked by the IR light (Liu et al., 2013) and changing the non-linear capacitance of prestin (Okunade and Santos-Sacchi, 2013). A combination of optoacoustic and direct hair cell exposure mechanisms are able to explain the results of this thesis and those previously published by other researchers.

Experiments of hybrid electro-optical stimulation, presented in this thesis, showed a moderate 8 – 10% reduction in electrical thresholds, even in profoundly deaf animals. However, it is unclear what the mechanism behind this enhancement is and if there is any benefit in spatial localisation of stimulation. Given the power requirements of currently available laser sources, it will require significant optimisation of the process to provide any clinical benefit.
Overall, from the experimental results presented here, it is unlikely that INS in an implantable device will show clinical benefits for profoundly deaf patients without significant further development. However, there remains potential for a device targeting inner hair cells for patients with partial hearing loss.

7.2 Future outlook

The thermal model of INS provides useful information about both the spatial and temporal behaviour of heat during INS. There are a number of limitations with the technique and results presented here. The quality of a model is only as good as the inputs, described by the computer science adage GIGO: “garbage in, garbage out”. The current Monte Carlo model is limited by the availability of scattering and absorption coefficients to calculate photon trajectories in tissue. Due to the relatively strong water absorption at the wavelengths of light typically used for INS, there has been little study of the scattering and absorption characteristics of different tissues. While the model predictions in Chapter 2 do not appear to be greatly affected by variations in the scattering coefficient within a reasonable range, the results would be more robust if experimental measurements of the optical properties were available for the tissues of interest to INS. Another limitation is the simple geometry typically used. For example, the model in this work has treated tissue regions as homogeneous slabs. While this provides a good first approximation, a more detailed understanding could be gained through use of more detailed structural models.

To further understand the biophysical mechanisms behind INS, the model could be extended to include simulated neuronal behaviour due to the shifts in temperature. Adding a neuronal response would provide a complete model, giving predictive powers from different input parameters, allowing for further optimisation of INS delivery techniques. Finally, additional comparisons between experimental results and the predictions of the model could be used to further refine the model, optimise experimental technique and assist in understanding the biophysical mechanisms behind INS.

Although this work strongly suggests that a response to INS in the cochlea requires functioning hair cells, more research is needed to understand the mechanism behind this dependence. Although a purely optoacoustic response can be generated in the cochlea with the same lasers as used for INS, hair cells in the beam path also appear to be excited. If inner hair cells are excitable due to exposure from infrared radiation, this could help to clarify the mechanism and lead to the development of implants to target this pathway. Experiments to assist clarifying the mechanism
of infrared stimulation include *in vitro* excitation of hair cells, both isolated and in tissue slices. Patients with residual activity in their inner hair cells could therefore have enhanced spatial selectivity though use of this technique.

Interactions between infrared light and hair cells may provide novel ways of interrogating their function, providing further insights to the mechanisms behind hearing. The authors of recent work showing that outer hair cells respond to rapid heating from infrared lasers have suggested that infrared lasers could provide a method of manipulating cochlear outer hair cell function *in vivo* (Okunade and Santos-Sacchi, 2013). Although it appears unlikely that INS will provide a revolutionary improvement to cochlear implant functionality, it may open the door to a number of interesting new scientific techniques. The emergence of more efficient laser sources in future may also help to promote these applications.


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