BIOPHYSICAL ASPECTS OF PHOTODYNAMIC THERAPY

Ronan Valentine

A Thesis Submitted for the Degree of PhD at the University of St. Andrews

2011

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Biophysical Aspects of Photodynamic Therapy

Thesis presented for the degree of

Doctor of Philosophy

to the University of St Andrews

by

Ronan Valentine

June 2011
Declarations

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Abstract

Photodynamic therapy (PDT) is a multimodality cancer treatment available for the palliation or eradication of systemic and cutaneous malignancies. In this thesis, the application of PDT is for the treatment of non-melanoma skin cancer (NMSC). While PDT has a well-documented track record, there are, at this time no significant indicators to suggest the superiority of one treatment regime over the next. The motivation for this work is to provide additional evidence pertaining to PDT treatment variables, and to assist in optimising PDT treatment regimes. One such variable is the treatment light dose. Determining the light dose more accurately would assist in optimising treatment schedules. Furthermore, choice of photosensitiser pro-drug type and application times still lack an evidence base.

To address issues concerning treatment parameters, fluorescence spectroscopy – a valuable optical diagnostic technique – was used. Monitoring the in vivo PpIX fluorescence and photobleaching during PDT was employed to provide information pertaining to the progression of treatment. This was demonstrated by performing a clinical study at the Photobiology Unit, Ninewells Hospital and Medical School, Dundee. Two different photosensitiser pro-drugs – either 5-aminolaevulinic acid (ALA) or its methyl ester (MAL) – were investigated and based on the fluorescence and pain data recorded both may be equally suitable for topical PDT.

During PDT, surface fluorescence is observed to diminish with time – due to photobleaching – although cancerous cells may continue to be destroyed deep down in the tissue. Therefore, it is difficult to ascertain what is happening at depth in the tumour. This raised the questions; How long after surface PpIX fluorescence has diminished is the PDT treatment still effective and to what depths below the surface is effective treatment provided? In order to address these important questions, a three-dimensional (3D) Monte Carlo radiation transfer (MCRT) model was used to compute the light dose and the \( ^1\text{O}_2 \) production within a tumour, and the PpIX fluorescence emission from the tumour. An implicit dosimetry approach based on a single parameter – fluorescence photobleaching – was used in order to determine the \( ^1\text{O}_2 \) generation, which is assumed to be related to tissue damage. Findings from our model recommended administering a larger treatment light dose, advocating an increase in the treatment time after surface PpIX fluorescence has diminished. This increase may ultimately assist in optimising PDT treatment regimes, particularly at depth within tumours.
Acknowledgements

I would like to begin by thanking Dr. Tom Brown who always offered me assistance and guidance. I greatly appreciated your supervision.

To Professor Harry Moseley; You have been an inspiration to me. I am sincerely grateful for all the opportunities you gave me during my PhD, particularly allowing me to attend and present at numerous conferences. Your support and confidence in me have been invaluable and will truly have a lasting effect.

For Dr. Kenny Wood; Thank you for providing me with the opportunity to undertake this PhD. I express my gratitude for your time, patience and support during the course of this research. Your enthusiasm has constantly motivated me and I’ll always value this experience.

Also, I would like to thank Dr. Sally Ibbotson for her assistance during my clinical study and throughout my entire PhD.

A big thank you goes to all the staff and technicians at the Photobiology Unit in Ninewells hospital; June, Andrea, Shelagh, Lynn, Leona, Faiza, Laura, Pat, Lesley, Nicki, Kim, Kevin, Clare, Julie, Sian, Lorraine, Carol and of course, Ewan. Thank you guys for being so kind and helpful.

I am very grateful to all the members of the W-squad; Chris, Klaus, Alex, Craig, Christine, Fiona, Dave and of course, Flavio. You have all assisted me so much and were always willing to help me solve my problems. Thank you guys for including me in your group!

For all my friends and colleagues, both in St. Andrews and Dundee. I will sincerely cherish the memories. Thank you very much.

To the Gardner family, June, John and Paula. You truly are fantastic people. Thank you all so very much for the past three months. I will never forget it.

I would like to thank my girlfriend, Claire for always helping me to see things from a different point of view. I think the world of you and I’m so very happy that we met.
Finally, my family. Words cannot express how much you all mean to me. Thank you Dad, Elaine, Paul and especially Mam – the much needed frequent phone calls! – for all your support, encouragement and love throughout the last three and a half years and always.

List of Publications

Refereed Journal Publications


Conference Presentations


*Laser Europe PDPDT Best Presentation Prize – Tarragona, Spain, May 2010.
List of Abbreviations

PDT Photodynamic Therapy
NMSC Nonmelanoma Skin Cancer
PD Photodiagnosis
UVR Ultraviolet Radiation
MM Malignant Melanomas
NIH National Institute of Health
BCC Basal Cell Carcinoma
SCC Squamous Cell Carcinoma
BD Bowen’s Disease
MMS Mohs Micrographic Surgery
5-FU 5-Fluorouracil
CR Complete Response
Hp Hematoporphyrin
HpD Hematoporphyrin Derivative
5-ALA/ALA 5-Aminolaevulinic Acid
Metvix/MAL Methyl Aminolevulinate
ALA-PDT 5-Aminolaevulinic Acid Photodynamic Therapy
MAL-PDT Methyl Aminolevulinate Photodynamic Therapy
sBCC superficial Basal Cell Carcinoma
AK Actinic Keratosis
nBCC nodular Basal Cell Carcinoma
PpIX Protoporphyrin IX
ROI Region of Interest
LD Light Dose
LED Light Emitting Diode
BPD Benzoporphyrin Derivative
AMD Age-related Macular Degeneration
mTHPC meso-TetraHydroxyPhenyl-Chlorine
AlSPc Aluminium Chlorophthalocyanine Sulfonate
Fe$^{2+}$ Ferrochelatase
ISC Intersystem Crossing
ROS Reactive Oxygen Species
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>IC</td>
<td>Internal Conversion</td>
</tr>
<tr>
<td>PB</td>
<td>Photobleaching</td>
</tr>
<tr>
<td>FD</td>
<td>Fluorescence Diagnosis</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide Adenine Dinucleotide</td>
</tr>
<tr>
<td>PDD</td>
<td>Photodynamic Dose</td>
</tr>
<tr>
<td>RTE</td>
<td>Radiative Transfer Equation</td>
</tr>
<tr>
<td>MCRT</td>
<td>Monte Carlo Radiation Transfer</td>
</tr>
<tr>
<td>SOLD</td>
<td>Singlet Oxygen Luminescence Dosimetry</td>
</tr>
<tr>
<td>OBS</td>
<td>Optical Biopsy System</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical Coherence Tomography</td>
</tr>
<tr>
<td>FI</td>
<td>Fluorescence Imaging</td>
</tr>
<tr>
<td>LCI</td>
<td>Low Coherence Interferometry</td>
</tr>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
</tr>
<tr>
<td>SLD</td>
<td>Superluminescent Diode</td>
</tr>
<tr>
<td>DRS</td>
<td>Diffuse Reflectance Spectroscopy</td>
</tr>
<tr>
<td>RS</td>
<td>Raman Spectroscopy</td>
</tr>
<tr>
<td>LIFS</td>
<td>Light-Induced Fluorescence Spectroscopy</td>
</tr>
<tr>
<td>UV-visible</td>
<td>UltraViolet-visible</td>
</tr>
<tr>
<td>USB</td>
<td>Universal Serial Bus</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-Noise Ratio</td>
</tr>
<tr>
<td>CFS</td>
<td>Coproporphyrin Fluorescence Standard</td>
</tr>
<tr>
<td>COV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full Width Half Maximum</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the Curve</td>
</tr>
<tr>
<td>MPS</td>
<td>Mean Pain Score</td>
</tr>
<tr>
<td>PDF</td>
<td>Probability Distribution Function</td>
</tr>
<tr>
<td>CDF</td>
<td>Cumulative Distribution Function</td>
</tr>
<tr>
<td>PDₜ</td>
<td>Threshold Photodynamic Dose</td>
</tr>
<tr>
<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>HeNe</td>
<td>Helium-Neon</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>AAPM</td>
<td>American Association of Physicists in Medicine</td>
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Chapter 1 – Introduction and Background

1.1 Thesis Outline and Objective
This thesis is predominantly concerned with topical photodynamic therapy (PDT). The presented work addresses variables in PDT treatment regimes for nonmelanoma skin cancer (NMSC) that exist, due to there being no clear evidence regarding optimal treatment parameters. Although PDT is widely accepted as an effective treatment for cancer indications, owing to its high response rates and excellent cosmetic outcome, it still, however, remains in the shadow of other treatment techniques, such as radiation therapy, chemotherapy and surgery. In the context of the clinical administration of PDT, the following quantities can be observed; the administered quantity of the exogenous photosensitiser pro-drug, the time delay between this pro-drug application and the onset of treatment irradiation, the wavelength of the incident light and the light dose. In order to fully optimise clinical PDT, new approaches must be taken to investigate treatments by means of further clinical and theoretical studies.

The intention of this chapter is to give an overview of PDT and the components that contribute to the research of this multimodality cancer treatment. Firstly, the history, basic principles, mechanism of action of PDT and PDT-induced pain will be introduced, followed by the role of photodiagnosis (PD) in PDT. Next, the problem of light transport in tissue and methods of addressing this will all be outlined. Finally, in view of the above, PDT dosimetry will be discussed.

1.2 Overview of Photodynamic Therapy (PDT)

1.2.1 PDT – Past, Present & Future
Skin, the largest organ of the human body is composed of three structural layers; the epidermis, dermis and subcutaneous layer. The skin functions as a body temperature regulator and a physical barrier to the environment by providing protection against, for example, harmful radiation [1]. In 1975, “skin typing” was conceived, which essentially classifies skin according to its reactivity to sunlight [2]. The six types of skin are listed in Table 1.1.
### Table 1.1 Fitzpatrick skin type classification

<table>
<thead>
<tr>
<th>Skin Type</th>
<th>Sunlight Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Always burns, never tans; White skin colour</td>
</tr>
<tr>
<td>II</td>
<td>Usually burns, minimal tanning; White skin colour</td>
</tr>
<tr>
<td>III</td>
<td>Sometimes burns, average tanning; White skin colour</td>
</tr>
<tr>
<td>IV</td>
<td>Slightly burns, above average tanning; Light Brown skin colour</td>
</tr>
<tr>
<td>V</td>
<td>Rarely burns, strong tanning; Brown skin colour</td>
</tr>
<tr>
<td>VI</td>
<td>Very rarely burns; Black skin colour</td>
</tr>
</tbody>
</table>

Chronic exposure of the skin to solar ultraviolet radiation (UVR) has been reported to cause DNA damage and immunosuppression [3], which is believed to be mainly responsible for the development of skin cancer [4]. Malignant melanomas (MM) have a lower incidence than NMSC but are very aggressive skin cancers, which can metastasise to other body sites and eventually result in death [5]. Alternatively, NMSC are less aggressive and not as life threatening, although they have the potential to cause disfigurement through tissue destruction and can also metastasise, if left untreated for long periods of time. The National Institute of Health (NIH) has estimated that there were more than 1,000,000 new cases of NMSC in the United States in 2010 [6], while the NHS estimate 100,000 new cases of NMSC in the UK every year [7]. NMSC, (including basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and squamous cell carcinoma in situ (Bowen’s disease [BD])) is the most common cancer in Caucasians [8].

There are several conventional therapeutic modalities, such as surgical excision (including mohs micrographic surgery [MMS]), cryotherapy, topical imiquimod and 5-fluorouracil (5-FU), which may be employed for the treatment of NMSC. While MMS offers a particularly high complete response (CR), PDT has also demonstrated a high CR in many clinical trials [9-18].

The administration of a photosensitiser followed by the irradiation of the target region of interest with light of an appropriate wavelength activates the photosensitiser [19]. The interaction between the excited photosensitiser and molecules can lead to the indirect or direct production of cytotoxic species, such as, radicals and singlet oxygen [20]. Singlet oxygen is believed to be the main cytotoxic agent involved in PDT [21].
Chapter 1 – Introduction and Background

PDT dates back as far as 1900, when a medical student, Oscar Raab, was the first to demonstrate – accidently – that the combination of acridine red and light elicited a detrimental effect on in vitro paramecium [22]. The transfer of energy from light to the chemical was significant for initiating toxic-induced effects to cells. In 1907, von Tappeiner and Jodlbauer coined the term “photodynamic reaction” and in 1913, Friedrich Meyer-Betz was the first to report human photosensitization by using 200 mg of a porphyrin photosensitiser – hematoporphyrin (Hp) [23]. Lipson is largely responsible for initiating the modern era of PDT when studies involving the photosensitiser, hematoporphyrin derivative (HpD) were performed in the 1960s [24,25]. Dougherty et al. [26] developed PDT further in 1975, by treating experimental animal tumours. The following year saw the first patient study of PDT using HpD in bladder cancer [27]. In 1978, Dougherty [28] first demonstrated the clinical effectiveness of PDT by performing treatments on 25 patients presenting with a total of 113 skin tumours. This human clinical trial achieved an 87 % CR and paved the way for many such clinical trials and studies. The concept of PDT employing 5-aminolaevulinic acid (5-ALA or simply ALA) was first introduced by Kennedy at al. [11]. Since then there has been a plethora of topical PDT studies and it is continually gaining clinical acceptance.

Today, ALA and its methyl ester (methyl aminolevulinate [MAL]) are the most commonly employed pro-drugs for PDT of NMSC. Robust, well-designed, clinical trials and studies have been performed, demonstrating the efficacy of ALA- and MAL-PDT, high long-term CR and ultimately providing informative guidelines for the use of PDT in the treatment of cutaneous disorders [9-18]. In 2007, international consensus guidelines were published in a landmark paper reporting on the clinical evidence of ALA- and MAL-PDT for oncologic indications, such as sBCC, BD, actinic keratosis (AK) and nodular basal cell carcinoma (nBCC) [29]. Also, Morton et al. [30] indicated that PDT with either ALA or MAL is efficacious for NMSCs. Table 1.2 lists the CR – for a follow-up period of at least three months – found through many clinical trials [11-18]. While PDT has been recognised as a reliable treatment modality and may be used as a first line therapy, the guidelines failed to specify the methodology – optimal treatment parameters – upon which the treatment is based, which is important for defining treatment outcomes. Furthermore, recurrence rates associated with PDT have been reported to be in the region of 20 %
at 2 years post-treatment in BD and at 2 – 3 years in sBCC [30]. Therefore, PDT has not yet reached its full potential in terms of clinical efficacy. Nevertheless, PDT has demonstrated consistently high CR along with superior cosmesis compared to many other treatment modalities [9-18].

Table 1.2 CR for varying oncologic indications reported by several clinical trials.

<table>
<thead>
<tr>
<th>Oncologic Indication</th>
<th>Complete Response (CR)</th>
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<tr>
<td></td>
<td>ALA-PDT</td>
</tr>
<tr>
<td>sBCC</td>
<td>90 %</td>
</tr>
<tr>
<td>BD</td>
<td>88 %</td>
</tr>
<tr>
<td>AK</td>
<td>87 %</td>
</tr>
<tr>
<td>nBCC</td>
<td>72 %</td>
</tr>
</tbody>
</table>

In an on-going effort to optimise PDT treatment regimes, which in turn, may facilitate improved clinical efficacy, several studies have measured the fluorescence of protoporphyrin IX (PpIX) accumulated in NMSC as a function of varying prodrug application times [31]. Again, by recording PpIX fluorescence, Moseley at al. [32], studied the local uptake of ALA following surface preparation of lesions by curettage. It has been reported that oxygenation may be maintained during light illumination, particularly if low fluence rates are used [33]. Therefore, reducing the photochemical depletion of oxygen during treatment may facilitate enhanced treatment outcomes. Consequently, the field of PDT research has seen recent advances with the introduction of low irradiance ambulatory PDT [34]. The intention in this case is to reduce higher conventional fluence rates with a view to optimising treatment outcomes while reducing pain. Furthermore, de Haas et al. [35] reported on the success of ALA-PDT when performed under a fractionated illumination scheme. They concluded that light fractionated ALA-PDT should be considered for the effective treatment of NMSC.

In the following sections, emphasis will be placed upon critical aspects of PDT that constitute a treatment protocol. To achieve improved clinical PDT treatment outcomes, it is necessary to obtain further knowledge pertaining to, for example, light dosimetry, choice of photosensitiser prodrugs, and also the characteristics of PpIX fluorescence and photobleaching detected from NMSC during PDT.
1.2.2 **Light-Tissue Interactions**

Light sources together with the delivery of light are important aspects in PDT [36]. Conventional lamps were the first light sources used in PDT. Lamps are generally employed for direct illumination, are relatively cheap and easy to use. Various types of lamps that have been used for clinical PDT are the tungsten filament quartz halogen lamp, metal halide lamp and the xenon arc lamp. These will be discussed further in Chapter 6. However, calculating the light dose delivered to the region of interest (ROI) is difficult for these broadband light sources [37]. Lasers are commonly employed owing to their single wavelength, ease of light dose calculation and ability to be coupled to optical fibres for internal and localised treatment. Argon lasers, dye lasers, solid state lasers and diode lasers may be used in clinical PDT [36]. The light dose (LD) in J/cm\(^2\) is used to describe light delivery during PDT and is defined as the skin surface irradiance, \(I\) , (W/cm\(^2\)) multiplied by the treatment time, \(t\), (seconds) [38].

\[
LD = I \times t
\]  

Typically, the irradiance should not exceed 150 mW/cm\(^2\) as this will avoid hyperthermic effects and light doses ranging between 37 – 540 J/cm\(^2\) have been used [39]. For PDT of NMSC however, a non-coherent light source – with a relatively narrow spectrum – such as a light emitting diode (LED) is usually chosen due to its reliability, cost, ease of use and similar efficacy to laser irradiation. The choice of light source is highly dependent upon the photosensitiser used, as an appropriate wavelength of light will activate the photosensitiser, leading to the formation of cytotoxic species resulting in highly specific tissue destruction [36]. The transport of light is a significant feature in PDT and is largely dependent on the composition of the medium through which it is passing. Therefore, light propagation in biological tissue may be characterised by tissue optical property parameters [40].

1.2.2.1 **Optical properties**

The interactions of light with biological tissue can cause light absorption and scattering as the photons propagate through tissue. The light-tissue interactions of
absorption are attributed to melanin and hemoglobin concentration, while lipids and collagen are responsible for optical scattering in tissue [41]. Scattering in tissue is either elastic, which results in no energy transfer between the incident photon and the scattering molecule or inelastic, which occurs when there is an energy transfer from such photons [42]. Tissue optical properties, such as the absorption coefficient, $\mu_a$, scattering coefficient, $\mu_s$, scattering anisotropy factor, $g$ and the refractive index, $n$ are responsible for the behaviour of light within tissue. The absorption coefficient, $\mu_a$ (cm$^{-1}$) and scattering coefficient, $\mu_s$ (cm$^{-1}$) are defined as the probability per unit path length that a photon will meet an absorption or scattering event, respectively; the refractive index, $n$, is defined as the ratio of the speed of light in a vacuum to the speed of light in a medium; and the scattering anisotropy factor, $g$, which is indicative of mean cosine ($\cos \theta$) of the photon scattering angle relative to the direction of the incident photons [40]. These parameters will be discussed in greater detail later in Chapter 4, Section 4.2.

Optical properties are wavelength dependent and therefore the wavelength of any light source determines how deep the light may penetrate into the tissue. Figure 1.1 depicts the absorption of light by various chromophores in tissue and the therapeutic window – between 600 and 1000 nm – which is a spectral region ideal for diagnostic and therapeutic medical applications, such as PDT and PD [43]. In the visible region, the depth of light penetration increases as the wavelength increases [44]. Wavelengths within this range penetrate the deepest into tissue, partly due to lower scattering but mainly because of lower absorption [45]. A comprehensive review of optical properties was undertaken by Cheong et al., in 1990 [46]. Light distributions in tissue are rather involved and can be modelled using methods that describe light transport in tissue. This will be discussed further in Section 1.4.
1.2.3 Photosensitisers

Ideal photosensitisers suitable for clinical PDT should have the following properties (a) have a strong absorbance at long clinically useful wavelengths of light allowing for deeper penetration into tissue; (b) selectively accumulate in the target tissue; (c) be efficiently eliminated from the body resulting with minimal toxicity; (d) become cytotoxic only when light is present; and (e) have a high quantum yield of the photochemical reaction resulting in singlet oxygen generation [47].

HpD – as mentioned earlier – is a porphyrin photosensitiser. It’s most active components, thought to be di-haematoporphyrin ethers and esters are commercially available under the name Photofrin®, which is commonly employed for the treatment of brain tumours [48], head and neck neoplasms [49] and bladder tumours [50]. Other porphyrin photosensitisers include benzoporphyrin derivative (BPD), available commercially as Verteporfin® and used for the treatment of age-related macular degeneration (AMD) [51]. Prolonged skin photosensitivity of several weeks is a major...
side-effect and a significant drawback to systemically applied photosensitisers such as Photofrin® and Verteporfin®. Finally, meso-tetrahydroxyphenyl-chlorine – mTHPC, Foscan® – may be employed for PDT of head and neck and oesophageal tumours, while aluminium chlorophthalocyanine sulfonate (AlSPc) is another photosensitiser [52].

ALA is a naturally occurring hydrophilic amino acid and is a precursor to PpIX [47]. MAL, the methyl ester of ALA – is the only licensed formulation for topical PDT in Europe. Due to its lipophilicity, MAL should theoretically be more specific and penetrate deeper into tumour tissue than ALA [53]. However, there is a relative paucity of comparative studies of efficacy and no convincing data to support this [54,55]. PpIX is an efficient endogenous porphyrin photosensitiser that occurs naturally in all mammalian cells but at low levels. After topical application of the prodrugs – ALA and/or MAL – to a skin lesion, uptake and conversion to PpIX occurs via the natural heme biosynthetic pathway as illustrated in Figure 1.2. The cycle begins with ALA synthesis from glycine and succinyl CoA, moves through a series enzyme regulated conversions eventually leading to the formation of PpIX. By means of the enzyme, ferrochelatase – Fe$^{2+}$ –, PpIX is finally converted to heme and the cycle starts over. However, when exogenous prodrugs are topically applied to skin lesions, ferrochelatase activity may be overwhelmed and the biosynthesis of heme is disrupted, resulting in the accumulation of PpIX in lesional cells [56]. Therefore, these rapidly proliferating cells produce increased levels of PpIX, as a result of the rate limiting activity of ferrochelatase. Furthermore, skin lesions present with a damaged keratin layer, which further supports the accumulation and selectivity of PpIX in lesional cells [57]. PpIX has been shown to preferentially accumulate in lesional cells more than in surrounding normal skin [58] and therefore is the basis upon which fluorescence detection and PDT for skin disorders operates. Interestingly, for topically applied photosensitisers, skin photosensitisation is in the order of 24 – 48 hours.
Figure 1.2 *The Natural Heme Biosynthetic Pathway.*

PpIX exhibits an absorption spectrum with five peaks. Figure 1.3 depicts the five absorption bands of PpIX. The highest and lowest absorption peaks are at approximately 405 and 630 nm, respectively, with 630 nm showing the best tissue penetration. Therefore, a trade-off exists between optimal absorption of light by the photosensitiser and optimal tissue penetration [59]. It has been reported that the penetrating depth of a photosensitiser into tissue is more of a limiting factor than the penetration of light [60].
1.2.4 Mechanism of Action

The combination of light, photosensitiser and tissue oxygenation yields photodynamic reactions responsible for tumour destruction. Figure 1.4 illustrates the Jablonski diagram showing the underlying photodynamic reactions associated with PDT. Firstly, a light photon of a specific wavelength is absorbed by an absorption band of a photosensitiser molecule, exciting the molecule from its ground singlet state, $S_0$, into a higher excited singlet state, $S_1$. Following this, the molecules can relax back down to the ground singlet state by emitting photons. This loss of energy is known as...
fluorescence. Alternatively through intersystem crossing (ISC) the molecule may cross into a excited triplet state, T_1. The lifetime of the molecule in the excited triplet state is longer than that achieved in the excited singlet state and thus the molecule is more likely to react with its surroundings [61].

Photodynamic reactions can be split into two processes, known as Type I and Type II reactions. Type I reactions involve the transfer of an electron or hydrogen atom from the excited triplet state photosensitiser molecule to a nearby molecule – such as a membrane lipid – generating radicals, which frequently react with oxygen leading to the formation of reactive oxygen species (ROS) [61]. Type II reactions occur when energy is transferred from the excited triplet state photosensitiser, T_1, to molecular oxygen, \(^3\)O_2, thus generating highly reactive singlet oxygen, \(^1\)O_2. \(^3\)O_2 is atypical, which has a ground triplet state, T_0, as opposed to a ground singlet state, S_0, which is characteristic of many molecules. It is believed that \(^1\)O_2 is the main cytotoxic agent involved in PDT [21]. PDT-induced tissue damage is reported to occur via three interdependent mechanisms. Firstly, direct effects – necrosis and apoptosis – are responsible for direct tumour destruction. Secondly, vascular effects may lead to vascular collapse and/or vascular leakage. Thirdly, an inflammatory response is induced in the tumour cells, whereby cytokines are released [62].
PDT-induced pain is a major side-effect of the treatment and is often regarded as the main acute adverse event experienced by patients during PDT of skin disorders [63]. Pain is often described by patients as an uncomfortable burning or stinging sensation felt during light irradiation in the treatment area. In fact, pain may continue after light irradiation and may last for up to several hours. Pain can be problematic during irradiation, as patients sometimes request a break, while others find it challenging to remain still making it difficult to ensure accurate alignment of the light source.
Factors influencing pain are not well defined and therefore the mechanism behind PDT-induced pain is unidentified [64]. However, it is believed to be due to nerve stimulation or damage by ROS [65]. Efforts have been made in treatment clinics in order to reduce pain sensations. Variations in pain experienced by patients during irradiation have made it difficult to accurately assess pain and therefore present an ongoing challenge.

1.3 Photodiagnosis (PD)

Histopathology can define the characteristic features of NMSC and is therefore the gold standard for diagnosing suspicious cells [66]. Histological diagnosis is important for treatment planning, which may be achieved through certain invasive techniques, such as 1) punch biopsy; 2) shave biopsy; 3) incisional biopsy; and 4) excisional biopsy. However, biopsies only provide local assessments of the diseased tissue state, along with a reduced patient experience and are expensive to carry out.

Interestingly, optical inspection of the suspicious tissue may be carried out by what is called photodiagnosis (PD). This will be discussed in detail in Chapter 2. Briefly, PD offers a non-invasive approach to differentiating cancerous cells from surrounding normal tissue for the treatment and eventual destruction of diseased tissue. There are a wide range of optical diagnostic techniques, with fluorescence spectroscopy recognised as a valuable optical technique that may be used clinically for the early detection and diagnosis of tumours and to assist in the monitoring of PDT treatments [67]. Fluorescence diagnosis (FD), which is a specific case of PD will be discussed in the following section.

1.3.1 Fluorescence Diagnosis (FD)

The phenomenon of fluorescence is caused by the irradiation of a molecule by light of a particular wavelength and the subsequent light emission of a – lower energy – longer wavelength. At the molecular level, in accordance to the Jablonski diagram – Figure 1.4 – an excited molecule rapidly returns from the $S_1$ to the $S_0$ by fluorescence emission [61]. Table 1.3 lists well known skin chromophores [68]. Fluorescence observed from naturally occurring endogenous skin chromophores – nicotinamide adenine dinucleotide (NADH), collagen and tryptophan – is known as
autofluorescence, which exhibits relatively broad spectral emission and has been used to discriminate normal skin tissue from NMSC [43].

Table 1.3 Pertinent skin chromophores responsible for tissue fluorescence signatures
Adapted from [68].

<table>
<thead>
<tr>
<th>Skin Chromophore</th>
<th>Absorption Spectral Range</th>
<th>Fluorescence</th>
<th>Absorption Maxima (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyhaemoglobin</td>
<td>UV-Visible</td>
<td>No</td>
<td>412, 542, 577</td>
</tr>
<tr>
<td>Deoxyhaemoglobin</td>
<td>UV-Visible</td>
<td>No</td>
<td>430, 555, 760</td>
</tr>
<tr>
<td>Melanin</td>
<td>UV-Visible</td>
<td>No</td>
<td>Increases with decreasing wavelengths</td>
</tr>
<tr>
<td>Water</td>
<td>IR-Long Visible</td>
<td>No</td>
<td>760, 900, 1250, 1400</td>
</tr>
<tr>
<td>Porphyrins</td>
<td>Visible</td>
<td>Yes</td>
<td>Ex: ~ 405 nm; Em: 630 nm</td>
</tr>
<tr>
<td>NADH</td>
<td>UV</td>
<td>Yes</td>
<td>Ex: ~ 350 nm; Em: 460 nm</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>UV</td>
<td>Yes</td>
<td>Ex: 295 nm; Em: 340-350 nm</td>
</tr>
<tr>
<td>Collagen</td>
<td>UV</td>
<td>Yes</td>
<td>Ex: 335, 370 nm; Em: 380, 460 nm</td>
</tr>
<tr>
<td>Elastin</td>
<td>UV-Visible</td>
<td>Yes</td>
<td>Ex: 420, 460 nm; Em: 500, 540 nm</td>
</tr>
</tbody>
</table>

Brancaleon et al. [69] have previously shown that in vivo autofluorescence of NMSC is different from that of normal tissue due to variations in tryptophan and dermal collagen crosslinks. Therefore, they suggest that endogenous fluorescence exhibited by NMSC may be used for non-invasive diagnostics and for detecting tumour margins. Fluorescence can indicate the amount of light energy absorbed in a photosensitiser. Therefore FD may be used as a tool for tissue diagnosis and also PDT dosimetry. Furthermore, FD may also improve knowledge of photosensitiser delivery [70]. Due to the selective build-up of PpIX in tumours, an in vivo fluorescence diagnostic technique – fluorescence spectroscopy – shows the preferential accumulation of PpIX in lesional cells. Selective PpIX accumulation in tumours
exhibits well-defined, intense characteristic fluorescence spectral peaks, which can delineate tumour margins, facilitate the early diagnosis of tumours and assist in the monitoring of PDT treatments [71]. Spectra of PpIX fluorescence and autofluorescence will be illustrated later in Chapter 2, Figure 2.11.

Typically, for FD PpIX is excited by blue light at approximately 400 nm, thereby causing it to emit a red fluorescence signature with a dominant peak at approximately 635 nm [72]. The fluorescence characteristics of PpIX can be used diagnostically, such as for NMSC. While for PDT, PpIX may be activated at around 630 nm and this is the preferred waveband for use in PDT due to its depth of tissue penetration [73]. Prodrug-induced PpIX generation depends on the enhanced retention at the site of application, the augmented rate of pro-drug uptake and also on the rate of enzymatic conversion of the pro-drug into PpIX [74]. The success of PDT may be limited by the transport and distribution of the prodrug in the tumour. While PDT is highly dependent on tissue biopsy and histopathology confirmations it requires tissue removal, whereas FD notably offers rapid real-time, non-invasive diagnostic information pertaining to the diseased state of tissue.

1.3.2 Fluorescence Photobleaching

The reduction in optical absorbance and/or fluorescence as the photosensitiser is photochemically destroyed by light may be described as photobleaching [75]. The photobleaching mechanism – Figure 1.4 – is assumed to rely on singlet oxygen [76] where reactions occur between the ground state photosensitiser and singlet oxygen causing irreversible photosensitiser destruction [77]. During PDT – light irradiation – fluorescence photobleaching occurs leading to a decrease in the observed PpIX fluorescence. The photobleaching of a photosensitiser during treatment is believed to be due to the cytotoxic agents – $^1\text{O}_2$ – that induce damage to the target tissue and in effect may be used for PDT dosimetry. The work of Wilson et al. [75] has shown that by employing a technique such as implicit dosimetry, fluorescence photobleaching may be used as a dose metric. Fluorescence photobleaching is a quantity that may indirectly predict the production of singlet oxygen. Fluorescence and photobleaching measurements can contribute significantly to the development of PDT dosimetry [78].

PpIX photobleaching was assumed to follow a first-order exponential decay.
where the PpIX concentration, \( C(x, y, z, t) \) decreased at a rate proportional to the local fluence rate, \( \Psi(x, y, z) \), as is shown in the following equation

\[
\frac{dC(x, y, z, t)}{dt} = -\frac{\Psi(x, y, z)}{\beta} C(x, y, z, t)
\]  

where \( \beta \) is the photobleaching decay constant, in J/cm\(^2\), \((x, y, z)\) represents the depth into the tissue and \( t \) the treatment time point.

Photobleaching as described by Equation 1.2 alters the spatial distribution of PpIX in the tissue. The fluence rate distribution decays with tissue depth. The top layers experience the highest fluence rate and therefore photobleach faster [76]. It was assumed that the PpIX concentration had a negligible effect on the absorption coefficient of the tissue therefore resulting in a time independent fluence rate distribution [79].

Robinson et al. [77], have suggested that a reduction in photosensitiser fluorescence caused by photobleaching is indicative of the photodynamic dose (PDD) administered to the tumour during PDT treatment. The local yield of \( ^1\text{O}_2 \) generation per unit volume of tumour tissue is assumed to be proportional to the number of photons absorbed by the photosensitizer per unit volume of tumour tissue and this may be used to define the PDD [76]. Therefore, monitoring \textit{in vivo} PpIX fluorescence and photobleaching during PDT – by means of photosensitiser fluorescence measurements – may provide information about the amount of photosensitiser in the tissue that has become photobleached during treatment, allowing the PDD to be inferred. The selective accumulation of PpIX in lesional cells, which in turn, promotes high PpIX levels, is required for FD and PDT. The photobleaching of PpIX fluorescence in lesional cells may facilitate a highly selective tumour treatment with normal tissue sparing, as less PpIX is expected to be present in the surrounding normal tissue. Fluorescence photobleaching has been widely used in studies to determine the efficacy of PDT treatments. Knowledge of the characteristics of ALA and MAL-induced PpIX fluorescence and photobleaching during PDT may, therefore,
assist in optimising PDT regimes. PDT dosimetry together with a description of implicit dosimetry will be reported in Section 1.5.

1.4 Light Propagation in Tissue
Understanding the interaction of light with tissue is fundamental to PD and PDT. The radiative transfer equation (RTE) can offer a practical description of photon propagation in scattering media, such as tissue [80]. However, several approximations have been postulated as it is difficult to solve the RTE exactly. The problem of calculating light distributions within tissues can be addressed via the Beer-Lambert law, the diffusion approximation and the Monte Carlo Radiation Transfer (MCRT) method. These mathematical approaches are based on the RTE and have been used to compute and characterise tissue optical properties from experimental measurements [81]. Once the optical properties of the tissue are known, the RTE can be used to compute the fluence rate, \( \psi \) in units \( \text{Wcm}^{-2} \) – defined in the forthcoming section – at any position for a given source specification. The fluence rate is an important treatment parameter used in PDT. Ultimately, these models compute the distribution of light as it passes through biological media [41] and can therefore predict how the light will travel into target regions, such as tumours. Precise knowledge of optical properties is essential and directly impacts on the accuracy of the mathematical models [46].

1.4.1 The Radiative Transfer Equation (RTE)
The radiative transfer equation (RTE) is associated with the theory of light transport in tissue and thus forms the basis to all light propagation models [46]. The transport of photons in highly scattering tissue may be described by the RTE. The propagation of individual photons through biological tissue is affected by scattering, absorption and emission. Pertinent parameters relating to the RTE will be described next. The RTE considers changes in the flow of energy due to traversing photons and is used to describe the balance of specific intensity within a tissue volume, which can be expressed as follows [82]

\[
\frac{1}{c} \frac{\partial I_{\nu}(\hat{r}, \hat{s}, t)}{\partial t} + \hat{s} \cdot \nabla I_{\nu}(\hat{r}, \hat{s}, t) = - (\mu_a + \mu_t) I_{\nu}(\hat{r}, \hat{s}, t) \\
+ \mu_t \int_{4\pi} P(\hat{s}, \hat{s}') I_{\nu}(\hat{r}, \hat{s}', t) d\Omega' + j_{\nu}(r, \hat{s}, t)
\] (1.3)
where $c$ is the speed of light in the medium, $I_{\nu}(r,\hat{s},t)$ is the specific intensity of light at position $r$ travelling in the direction $\hat{s}$ in units $Wcm^{-2}sr^{-1}Hz^{-1}$, $\hat{s}$ is the directional unit vector, $P(\hat{s},\hat{s}')$ is the scattering phase function, which describes the angular distribution of a single scattering event, $d\Omega$ is the differential solid angle and $j_{\nu}(r,\hat{s},t)$ – volume emissivity – in units $Wcm^{-3}sr^{-1}$, denotes a source term, which represents a local source of photons. The RTE terms physically represent the following:

$$\frac{1}{c}\frac{\partial I_{\nu}(r,\hat{s},t)}{\partial t} + \hat{s} \cdot \nabla I_{\nu}(r,\hat{s},t) \rightarrow \text{Difference between the flow of energy entering the volume and exiting the volume per unit time.}$$

$$(\mu_a + \mu_s) I_{\nu}(r,\hat{s},t) \rightarrow \text{Removal of energy from the beam of photons due to scattering and absorption (sink term).}$$

$$\mu_s \int_{4\pi} P(\hat{s},\hat{s}') I_{\nu}(r,\hat{s}',t) d\Omega' \rightarrow \text{Increase in energy due to scattering of photons from all directions } \hat{s}' \text{ into the direction } \hat{s} \text{ (source term).}$$

$$j_{\nu}(r,\hat{s},t) \rightarrow \text{Increase in energy due to a source of photons within the volume (source term).}$$

The fluence rate, $\psi$, is defined as the energy flow per unit area per unit time irrespective of flow direction and is expressed as the specific intensity integrated over the entire $4\pi$ solid angle at a location

$$\psi(r,t) = \int_{4\pi} I_{\nu}(r,\hat{s},t) d\Omega d\nu \quad (1.4)$$
The spectral radiance – specific intensity – $I_{sr}(r, \hat{s}, t)$, is defined as the radiant energy, $dE_{sr}$, passing through a unit surface area $dA$ at an angle $\theta$ to the surface normal within a solid angle $d\Omega$ in a frequency range $d\nu$ in time $dt$ in units $W cm^{-2} sr^{-1} Hz^{-1}$.

$$dE_{sr} = I_{sr}(r, \hat{s}, t) \cos \theta dA d\Omega d\nu dt$$  \hspace{1cm} (1.5)

**Figure 1.5** Radiant energy transported through a unit surface area, $dA$ within a solid angle, $d\Omega$ [83].
1.4.2 Methods for Solving the Radiative Transfer Equation

In this section, the propagation of light in turbid media will be described by three different approaches. These light propagation models attempt to solve the RTE and essentially describe the distribution of light in tissue.

### 1.4.2.1 Beer-Lambert law

This is a simple solution to the RTE and may be implemented when no scattering exists. Therefore absorption events are dominant. However, tissue is highly scattering and renders this theory inappropriate for modelling light transport in tissue. Here, the incident specific intensity, $I_v(0)$ is attenuated exponentially to the transmitted specific intensity, $I_v(z)$ due to the thickness of the slab and the absorption coefficient. The Beer-Lambert law may be expressed as follows

$$I_v(z) = I_v(0)e^{-(\mu_a)z}$$

(1.6)

where $\mu_a z$ is the optical depth of the slab through which the light passes.

### 1.4.2.2 Diffusion Approximation

The diffusion approximation offers an approximate solution to the RTE. It assumes isotropic scattering within the medium and best results are achieved when scattering is much larger than absorption [82]. Although the diffusion approximation has been widely used in medical applications it has serious limitations. This approach is valid only when scattering is assumed to be much greater than absorption. The accuracy of the diffusion approximation is affected by anisotropic scattering, which is typical of highly forward scattering biological tissue. In tissue regions close to the surface and the illumination source, the diffusion approximation breaks down due to the scattering being highly anisotropic. As the light has a mainly forward scattering direction the assumption of isotropic scattering is invalid. Furthermore, the angular distribution of the light is poorly approximated by the diffusion approximation [84]. The scattering anisotropy factor, $g$, as defined earlier in Section 1.2.2 may be given varying values, where $g = 0$ represents isotropic scattering, $g = 0.5$ denotes forward scattering and
g = 0.7 is indicative of more forward directed scattering. These values of g are illustrated in Figure 1.6. A further description of g is given in Chapter 4, Section 4.2.

![Figure 1.6 Varying values of the scattering anisotropy factor, g.](image)

### 1.4.2.3 Monte Carlo Radiation Transfer (MCRT) Method

The Monte Carlo Radiation Transfer (MCRT) method is a numerical technique that solves the RTE in order to compute the distribution of light in tissue. It offers information about the trajectories of individual photons through a medium, which builds up an accurate depiction of where and how the light traversed the medium [85]. Advantages of the MCRT method over the above mentioned models are listed in Table 1.4. Consequently, this mathematical model is often chosen to simulate light transport and compute the light dose administered to structures similar to those observed in PDT [86]. Unlike the diffusion approximation it yields accurate results near surfaces and for complicated geometries over a wide range of tissue optical properties. Furthermore, it can be used to compute the light dose and/or acquire the optical properties of tissue. Therefore, it can be effectively used for describing light propagation in tissue, particularly for PDT applications. A detailed description of the MCRT method will be outlined in Chapter 4.
Table 1.4 A comparison of the qualities of each different approach for solving the RTE.

<table>
<thead>
<tr>
<th>Model Qualities</th>
<th>Mathematical Model Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beer-Lambert Law</td>
</tr>
<tr>
<td></td>
<td>Diffusion Approximation</td>
</tr>
<tr>
<td></td>
<td>Monte Carlo Radiation Transfer</td>
</tr>
<tr>
<td></td>
<td>(MCRT) Method</td>
</tr>
<tr>
<td>Absorption</td>
<td>Yes</td>
</tr>
<tr>
<td>Isotropic Scattering ($g = 0$)</td>
<td>No</td>
</tr>
<tr>
<td>Anisotropic Scattering ($g \neq 0$)</td>
<td>No</td>
</tr>
<tr>
<td>($\mu_a \square \mu_s$) Limitation</td>
<td>No</td>
</tr>
<tr>
<td>Mismatched Boundaries</td>
<td>No</td>
</tr>
<tr>
<td>PD &amp; PDT Suitability</td>
<td>LOW</td>
</tr>
<tr>
<td></td>
<td>MODERATE</td>
</tr>
<tr>
<td></td>
<td>HIGH</td>
</tr>
</tbody>
</table>

1.5 PDT Dosimetry
A successful PDT treatment relies on optimal reactions occurring between the three critical PDT components; light, photosensitiser and oxygen. PDT dosimetry is complicated due to the dynamic interactions of these three treatment factors, which were highlighted through the mechanism of PDT as illustrated in Figure 1.4. Therefore, calculating the PDD is not trivial. In fact, there is no exact widely accepted definition of dose and this together with no clear concurrence on measuring the dose and relating it to tissue response has made dosimetry in PDT rather challenging [87]. However, the $^1O_2$ yield during a PDT treatment may be used as a measure of the dose, as Weishaupt et al. [21], suggested that PDT biological damage is related to the $^1O_2$ yield. In keeping with this, Wilson et al. [75], characterised and described different PDT dosimetry models, where a specific dose metric was used in order to predict tissue damage;

1) Explicit Dosimetry – relies on the continuous detection of each interdependent component – light, photosensitiser and oxygen – individually during a PDT treatment. The dose metric is defined by the combination of these three different parameters. The light dose – as defined in Section 1.2.2 – may be measured by calculating the surface irradianence, while the photosensitiser concentration in the target
tissue may be quantified through optical spectroscopy via fluorescence [88]. Oxygen measurements have been performed in tissue. However, it is technically difficult to carry out in vivo tissue oxygen measurements and therefore in the clinical setting the product of the photosensitiser concentration and the light fluence is computed and used to predict the PDT effect [87]. This explicit dosimetry approach is challenging, as it requires the measurement of the different treatment factors, which through their interdependency will impact on each other.

2) Direct dosimetry – depends on the detection and measurement of $^1\text{O}_2$ during treatment. This may be achieved through $^1\text{O}_2$ luminescence dosimetry (SOLD), which detects $^1\text{O}_2$ luminescence at 1270 nm and in doing so can quantify the amount of $^1\text{O}_2$ generated. Therefore, the $^1\text{O}_2$ is itself the dose metric. Although SOLD has not yet been adapted for the clinical setting, efforts are on-going in an attempt to bring this technique into clinical use. This is a promising technique, which employs the measurement of a single parameter. Neidre et al. [89] demonstrated in vivo a link between the photobiological response and $^1\text{O}_2$ generation.

3) Implicit Dosimetry. The difficulty of measuring each treatment component has been highlighted with explicit dosimetry. Therefore, implicit dosimetry incorporating all treatment factors was developed. This dosimetry approach uses a surrogate measure – fluorescence photobleaching of the photosensitiser – during treatment to predict the biological response. It relies on a single parameter – fluorescence photobleaching – to predict the production of $^1\text{O}_2$. Oxygen is believed to be responsible, in part for the consumption of the photosensitiser during PDT. In vivo studies have shown a positive correlation between PpIX photobleaching and tissue damage [77]. Therefore photobleaching – as defined in Section 1.3.2 – is assumed to be primarily mediated by $^1\text{O}_2$ [88], and may be used as a dose metric. Farrell et al. [76] assumed that photosensitiser photobleaching depended only on the photosensitiser concentration and the fluence rate. Therefore the rate of photosensitiser photobleaching – rate of change of the photosensitiser concentration – followed an exponential decay with respect to the light dose. This can be easily and effectively implemented into a treatment protocol, via fluorescence measurements, which can ultimately be used to predict the treatment outcome. This has enabled implicit dosimetry to become one of the most widely used
Chapter 1 – Introduction and Background

dosimetry models for PDT. A clinical application of implicit dosimetry will be presented thoroughly in Chapter 5.

1.6 Synopsis
Chapter 2 compares various optical diagnostic techniques used for the identification and characterisation of cancers, which will ultimately lead to cancer treatment. Chapter 3 discusses a clinical PDT audit, which was performed at Ninewells Hospital, Dundee with the aim to improve knowledge of photosensitiser fluorescence and photobleaching characteristics, for the optimisation of PDT treatment regimes for NMSC. Chapter 4 describes the frequently used MCRT method for modelling light propagation in biological tissue. Chapter 5 is a MCRT study pertaining to PDT, which essentially incorporates some of the clinical results presented in Chapter 3 for the purpose of guidance. Finally, using the MCRT method again, PDT light sources were modelled and their respective distributions of light within a tumour during PDT simulated and presented in Chapter 6.

1.7 Conclusion
This chapter has reviewed and reported on the current status of clinical PDT in the context of – mostly – dermatology. Although MMS is often used for the treatment of skin cancer, PDT is becoming a popular non-invasive therapy among clinicians, owing to fewer side effects and excellent cosmetic outcomes [29,30]. In fact, PDT is the treatment of choice for superficial NMSC. However, there is a clinical need for further optimisation of PDT, particularly when assessing treatment outcomes at long-term follow-up [30]. The underlying features of PDT have been described and the principles upon which it operates presented. Understanding the fluorescence and photobleaching kinetics of PpIX may be used to describe the dynamic PDT interactions, which occur between light, oxygen and photosensitiser. This may assist in optimising PDT treatment regimes. Furthermore, the transport of light through tissue is an important aspect of PDT, which can be effectively assessed with light transport models. Accurate knowledge of the skin surface irradiance is a prerequisite for PDT treatments. In an effort to optimise PDT treatment regimes, the work presented in this thesis has sought to combine both the clinical and theoretical aspects of PDT. Emphasis has been placed on implicit dosimetry, which is important for
defining optimal treatment outcomes [75]. Knowledge of biophysics and photobiology with respect to PDT dosimetry may allow accurate calculation of the PDT dose administered to a tumour in vivo [87].

Optimal PDT treatment regimes would provide minimal damage to normal skin while delivering an effective therapeutic result to diseased skin. Further in vivo studies are required and new provisions undertaken in order to further knowledge pertaining to PpIX fluorescence photobleaching in clinical PDT of NMSC. Also, modelling the transport of light in tissue and calculations of light distribution based on the RTE, which require knowledge of the absorption and scattering coefficients and the anisotropy factor can assist in optimising PDT treatment regimes.
1.8 References


Report No. 88, American Association of Physicists in Medicine, Medical Physics Publishing.


Chapter 2 – Photodiagnosis in Topical Photodynamic Therapy

2.1 Introduction
Early detection and diagnosis of cancers is essential for safe and effective treatments, along with reduced complications [1]. Photodiagnosis (PD) may be used to identify and characterise cancers, which should assist in optimising therapeutic efficacy. The potential of PD has, therefore, stimulated the development of many optical diagnostic techniques, which are commonly used in biomedical research and for applications in routine clinical practice. In this chapter, a range of optical diagnostic techniques will be discussed, highlighting their operating principles, strengths and weaknesses, and clinical applications. The term “optical biopsy”, which refers to a diagnosis of the diseased state of tissue based on in situ optical measurements [2], will be introduced with the main topic focusing on fluorescence. An optical biopsy system (OBS) – that is frequently used as part of the Scottish photodynamic therapy service in the Photobiology unit at Ninewells Hospital and Medical School, Dundee – operating on the principle of fluorescence spectroscopy will be described. Accurate and routine measurements of this system were employed ensuring system reproducibility and consistency, which is important for converting measured fluorescence spectral data to quantitative, physical information. OBS spectral data illustrating fluorescence as a function of wavelength, together with an analysis of photobleaching is presented. A comparison between the OBS and the routinely used Woods lamp is mentioned. The main focus of this chapter is the reliable detection of fluorescence from the OBS, which is of particular interest to clinical practice where patients receive PDT. Fluorescence can be observed from different cohorts of patients and therefore is at the centre of research from both a diagnostic and therapeutic standpoint.

2.2 Optical Diagnostic Techniques
Diagnosis is recognised as an integral part of any treatment modality and histopathology is accepted as the gold standard of cancer diagnosis [3]. Although biopsies – such as those mentioned in Chapter 1, Section 1.3 – offer a wealth of information at a localised point, there is limited information in terms of real-time monitoring of PDT treatments. Alternatively, optical diagnostic techniques can be practically applied to guide and monitor PDT treatments in clinical settings, which
potentially provide a higher predictive accuracy in locating optimal biopsy sites and assist in defining surgical margins for tumour excision. Rather than requiring tissue excision, optical diagnosis depends on the interaction of optical radiation with biological tissue, which is advantageous insofar as it offers real time, non-invasive in vivo tissue diagnostic signatures. Figure 2.1 illustrates pertinent light tissue interactions, which are the crux of optical diagnostic techniques.

Figure 2.1 Light-tissue interactions upon which optical diagnostic techniques operate.

PD may potentially offer earlier diagnosis and immediate treatment, while reducing patient waiting times, impacting positively on their well-being both physically and mentally. These non-intrusive techniques provide quantitative information relating to the state of the tissue, without the need for invasive tissue removal [4]. Therefore, they are emerging as powerful tools to non-invasively obtain morphological and
biochemical information of biological tissue [5]. Optical investigations of tissue may be performed through either the construction of an image – imaging – or spectroscopy, by producing a spectrum. Some interesting imaging techniques will be outlined next.

2.2.1 Optical Imaging

The evolution of technology has given impetus to optical imaging, which is a valuable tool for the diagnosis and treatment of cancer. Generating and measuring an optical response in tissue is desired in order to obtain specific information pertaining to the diseased state of that particular tissue. The visual representation of an optical response enables the tissue to be interrogated with respect to cellular organisation and structure. In vivo optical imaging has the potential to negate invasive measures such as surgery, which is commonly advocated in many PDT centres. Imaging systems can illuminate large tissue surface areas and therefore can provide pertinent information at numerous locations over the total area for a single image acquisition. Optical coherence tomography (OCT) and fluorescence imaging (FI) are optical imaging techniques, which provide diagnostic information on the spatial distribution and localisation of tumours [6].

2.2.1.1 Optical Coherence Tomography (OCT)

Optical coherence tomography (OCT) is a promising non-invasive optical imaging technique operating on the principle of low coherence interferometry (LCI), which produces (2D) two-dimensional cross-sectional images [7]. It offers morphological information pertaining to the tissue under investigation. Typically a light source – superluminescent diode (SLD) – with a short coherence length and a broad spectral bandwidth is used. The power from this light source is split into a sample wave and a reference wave. Light reflected by the sample, i.e., tissue, is combined to the light reflected by the reference mirror and differences in their respective pathlengths allow reconstruction of tissue reflectivity to represent depth [8]. The position of the mirror can be moved, which offers a measure of depth within the tissue from where the reflected signal came from [9]. For OCT, near-infrared electromagnetic radiation is employed while multiple LCI scans are acquired, which generate a 2D optical image. OCT has been widely used in studies of the human eye [10]. However, it is an
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emerging imaging technique that may be used for skin studies. Cross sectional anatomy imaging of skin tissue can be performed on a micrometer scale, with high spatial resolution providing instant diagnostic information pertaining to the region of interest [11]. There are several reports on OCT and its application to PDT for skin cancer; Hamdoon et al. [12], have investigated OCT in lesion mapping, assisting in the correct delivery of PDT and monitoring the outcome. Results from this study indicate that OCT-guided PDT is a promising procedure, which attempts to efficiently discriminate between tumour and non-tumour margins, while monitoring and assessing the healing process. Gambichler et al. [13] reported that OCT images were capable of visualising altered skin architecture pertaining to BCCs. Furthermore, features that were observed only in images of lesional skin correlated well with corresponding sections of biopsy samples. While OCT has exceptionally high resolution, it has a limited penetration/detection depth of 1 – 1.5 mm; which is typical for OCT of skin tumours [10]. Further limitations of OCT include restricted sampled field sizes and multiple scans are necessary in order to acquire images. The practical application of OCT to the clinical environment is limited when compared to other optical diagnostic techniques particularly those involved in FD, such as fluorescence spectroscopy and fluorescence imaging. Therefore OCT is rarely used in PDT. Moreover, unlike OCT, FD may also be applied to monitor PDT treatment efficacy and investigate the rate change of fluorescence during and after treatment.

2.2.1.2 Fluorescence Imaging (FI)

Fluorescence imaging (FI) is based on the principle of detecting fluorescence from endogenous species, such as collagen, tryptophan, NADH and PpIX. The detection of fluorescence finds its origins as far back as 1924, when fluorescence emission from tumours under Woods light exposure was reported [14]. In vivo fluorescence imaging measurements involve illumination of a significant area of tissue surface and collection of the emitted light with a filtered camera for spectral discrimination [3]. When the tissue surface is imaged there is potential to extract relevant diagnostic information using fluorescence, which can identify precancerous and cancerous lesions, define lesion margins and guide localised treatment [15]. Fluorescence differences observed between adjacent tissues provide a description about the biochemical state of the tissue and the changes associated with disease development.
The advent of fluorescence biochemical markers has greatly assisted in optical non-invasive tumour detection and diagnosis [16]. As mentioned earlier in Chapter 1, Section 1.2.3 the photosensitiser pro-drug – ALA – is responsible for causing the build up of the potent photosensitiser, PpIX in tissue. Consequently, the fluorescence signal will be increased dramatically particularly when compared with intrinsic autofluorescence signals. The spatial distribution of fluorescence intensity levels can be recorded and subsequently report on specific locations of the lesion. Fluorescence images provide a visual inspection of the tissue, which allows for the demarcation between non-lesional and lesional tissue sites with high sensitivity. By combining an excitation light source together with a multispectral imaging system consisting of an intensified camera, image splitter and an image intensifier – to amplify the faint fluorescence signal – it is possible to not only obtain a visual inspection but also acquire quantitative fluorescence measurements [17]. Ultimately, fluorescence imaging may be used for dosimetry in PDT, guide tissue biopsy and surgical resection as it offers diagnostic feedback regarding a large area of suspicious tissue [1].

2.2.2 Spectroscopy Methods
Optical spectroscopy is an important diagnostic technique, where optical radiation is employed for the non-invasive, real-time diagnosis of disease. Quantitative optical spectroscopy offers meaningful knowledge on tissue optical properties and therefore provides information relating to changes exhibited by tissues as they transition to varying cancers. The interaction of light with turbid biological tissue allows for quantitative biochemical and morphological information about tissue to be ascertained [18]. Light-tissue interactions, such as absorption, scattering, reflection and fluorescence are responsible for tissue characterisation, which is pertinent when employing optical spectroscopy [19]. There are beneficial aspects associated with these techniques, such as they are quantitative, fast, non-invasive, have high sensitivity and can be used under clinical conditions [20]. Various types of optical spectroscopy exist and some interesting methods are discussed below. In these procedures, light is used to interrogate the spatial variations in optical properties associated with tissues. By obtaining information on reflection, scattering, absorption and fluorescence, it is possible to generate data pertaining to the morphology and composition of tissues [6].
2.2.2.1 Diffuse Reflectance Spectroscopy (DRS)

Elastic scattering results in the energy of an incident photon being unaltered after colliding with a molecule and the resultant scattered photon has the same frequency as the incident photon [6]. Diffuse reflectance spectroscopy (DRS) is an optical spectroscopy technique that offers a non-invasive real time assessment of tissue pathology. Fibre optic probes are employed, which ensure optical contact with the tissue under investigation. DRS using spectral measurements of elastically scattered light is sensitive to the absorption and scattering characteristics of tissue over a range of wavelengths and thus can detect alterations of specific structures within tissue [21]. Scattering in tissue results from tissue morphology such as structures like nuclei, mitochondria and gradients in the refractive index. [22], epithelial thickness and collagen content, while absorption is mainly affected by changes in blood and oxygenation concentration [23]. Therefore, this technique is sensitive to structural changes – such as mitochondrial size and nuclear to cytoplasm ratio – at the cellular and sub-cellular level, which occurs when tissues transition from normal to pre-malignant and malignant states [11]. The potential of DRS to detect and diagnose tissue pathology arises from its ability to demonstrate changes in the scattering spectrum pertaining to morphological changes in the tissue. Therefore, DRS spectral signatures offer site-specific measurements, which can describe the state of the tissue under examination. Typically, a broadband light source is used for illumination and the reflected light is collected as a spectrum [23]. Light is delivered – by an optical fibre – to the suspicious tissue and the reflected light is gathered by a collection of optical fibres. When light impinges on tissue it undergoes multiple elastic scattering and absorption. As the photons traverse the tissue, further scattering and absorption can occur before exiting at the surface, where they offer diffuse reflectance spectra carrying quantitative information about the tissue optical properties, structure and composition [24]. These spectra are used to extract information pertaining to the light scattering and absorption properties of the tissue. DRS may be used to provide quantitative measurements of wavelength dependent absorption and scattering coefficients relating to tissue morphology; knowledge of which are important for PDT [25]. By combining both DRS and light-induced fluorescence spectroscopy (LIFS) together, it is possible to provide complementary information relating to tissue morphology and biochemical composition [26]. Mourant et al. have demonstrated the
feasibility of DRS for the optical biopsy of sampled tissue [27]. This \textit{in vivo} spectroscopic technique can be used for noninvasive, real time assessment of information relating to the structure and composition of invading tumours.

\textbf{2.2.2.2 Raman Spectroscopy (RS)}

Raman spectroscopy is a type of vibrational spectroscopy, which is used to probe the vibrational energy levels of tissue molecules [28]. As a consequence of the molecular vibrations of the illumination sample, there is a difference between the energy of an incident photon and the scattered photon. This inelastic scattering generally causes a change in photon energy, which gives information on the vibrational modes of the analyte [29]. The inelastic collision of an incident photon with a molecule generates an energy exchange and a small shift in the frequency of the scattered photon. This is known as the Raman shift and is specific to the biochemical constituents in tissue that produce the scattering [30]. The Raman shift is typically described in terms of wavenumber (cm$^{-1}$). Raman spectroscopy takes advantage of inelastic light scattering and facilitates biochemical fingerprints of biological tissue under investigation to be recorded [31]. Raman spectra exhibit relatively narrow peaks, which are sensitive to molecular composition and structure, and when grouped together generate a spectral fingerprint of information pertaining to a molecule of interest [32]. The majority of biological molecules – proteins, nucleic acids, lipids and water – exhibit Raman scattering each with their own characteristic fingerprint. Spectral changes give important information about the biochemical alterations in tissues. Therefore, it is possible to detect specific molecular and biochemical changes that accompany the onset of disease [33]. Raman scattering may be referred to as Stokes Raman scattering or anti-Stokes Raman scattering. If a scattered photon has a lower energy than the incident photon, this is referred to as Stokes scatter. Conversely, if a scattered photon has a higher energy than the incident photon, this is known as anti-Stokes scatter. Although Raman spectroscopy offers more specific information relating to tissue than fluorescence, it is not appropriate for all clinical diagnostic applications. Raman scattering is an inherently weak process and the intensity of the Raman signal is typically $10^{-9}$ to $10^{-6}$ of the elastic Rayleigh scattering background [34]. These signals must compete with strong fluorescence signals produced by wavelengths in the visible region of the electromagnetic spectrum. Therefore, the high fluorescence background
from biological tissues can act to overwhelm the Raman signals and therefore limits the measurement of Raman spectra from tissues [28]. Furthermore, complicated and expensive instrumentation is required for tissue characterisation and clinical use. However, near infra-red (NIR) Raman spectroscopy has been employed as this reduces fluorescence emission and increases the penetration depth of the light. For example, it has been shown that performing Raman spectroscopy at 830 nm was optimal for esophageal tissues [35]. Moreover, Raman spectroscopy – a potentially powerful technique with high sensitivity – has been used to investigate the molecular and biochemical alterations associated with diseased tissue in organs such as the skin [36], larynx [37], head and neck [11] and epithelial pre-cancers and cancers [38].

2.2.2.3 Absorption Spectroscopy (AS)

The absorption of light by a photosensitiser can be revealed by an absorption spectrum, which is detected by absorption spectroscopy [6]. The Beer-Lambert law as discussed in Chapter 1, Section 1.4.2.1 may be used to relate the absorption to the concentration of a species, i.e., photosensitiser. Determining the in vivo absorption spectrum for a photosensitiser allows the precise wavelength of the treatment light to be chosen [39]. By performing this optical method, it is therefore possible to estimate the concentration of a photosensitiser within tissue [40]. Knowledge of photosensitiser concentration, which may be ascertained from an absorption spectrum, is important for PDT dosimetry and achieving optimal treatment efficacy. The absorption coefficient can be determined over a range of wavelengths so that the characteristic peaks of a photosensitiser can be distinguished from absorption due to endogenous tissue. Furthermore, absorption spectroscopy offers information relating to tissue absorbers, such as oxy- and deoxy-haemoglobin in tissue [41]. While this spectroscopic technique can describe concentration dependent variations in absorption spectra allowing for increased selectivity for PDT treatments, it presents several disadvantages when compared to fluorescence spectroscopy. The sensitivity of absorption spectroscopy is lower than that achieved with fluorescence spectroscopy. Furthermore, biological tissue is highly scattering and therefore the scattered light can negatively impact on absorption measurements [42].
2.2.2.4 Light-Induced Fluorescence Spectroscopy (LIFS)

FD may be carried out quite effectively within the clinical environment through the use of light-induced fluorescence spectroscopy (LIFS). LIFS or simply fluorescence spectroscopy delivers excitation light to a tissue site and consequently measures the intensity of the fluorescence light at longer emission wavelengths. The use of fluorescence spectroscopy to discriminate normal and malignant tissue was reported as early as 1965 [43]. Alfano et al. were among the first to investigate tumour autofluorescence by detecting different naturally occurring fluorophores [44]. LIFS is commonly employed for tissue diagnostics and is based on the detection of characteristic fluorescence signatures from tissue fluorophores. It provides information on photosensitiser uptake and may be used as a tool for tissue characterisation, demarcation and diagnosis of premalignant and malignant skin lesions [1]. A narrow spectral band of incident light – narrowband laser – may excite a fluorophore – endogenous or exogenous – and result in an emerging fluorescence spectrum. The choice of excitation wavelength is important in relation to the fluorescence emission spectrum, as fluorophores absorb at specific wavelengths, which in turn facilitate their excitation and subsequent fluorescence emission. Similar to other optical techniques, this type of optical spectroscopy commonly employs fibre optic probes – placed in contact with the tissue surface – which deliver excitation light to a tissue site. It is possible to perform fluorescence spectroscopy with a single fibre, which is used for both light excitation and collection [45]. The end-point for this technique is to collect a fluorescence signal. Optical biopsy aims to replace unnecessary invasive diagnostic techniques with the spectral analysis of tissue recorded in vivo, in situ and in real time. Therefore, the concept of optical biopsy is one where diagnosis is based on performing optical measurements without tissue excision for histopathological examination.

In contrast to fluorescence imaging, tissue fluorescence may be measured in vivo by point-monitoring at a single site offering detailed spectroscopic information for one specific tissue site. The measured fluorescence signals contain information pertaining to the concentration and distribution of a fluorophore. Fluorescence is sensitive to the biochemical composition of the investigated tissue and these spectral signatures provide information relating to the biochemical state of the tissue [2]. Detecting the fluorescence emission from externally administered photosensitiser pro-
drugs – which accumulate preferentially in diseased tissue – can yield large fluorescence signals and have a positive impact on the diagnostic procedure [46]. Measurements of fluorescence from photosensitisers exhibit emission peaks, which can be attributed to porphyrins [47]. Therefore, PpIX fluorescence as a biochemical marker is an important indicator of the diseased state of the tissue. The concentration and selective accumulation of PpIX in tumours are important for optimal PDT treatments. Golub et al. employed in vivo surface-detected fluorescence measurements in order to obtain information relating to the pharmacokinetics of PpIX accumulation in skin lesions [48]. Investigating PpIX fluorescence has the potential to optimise treatment parameters and monitor the drug during treatment [49]. LIFS provides a quantifiable measure of PpIX fluorescence, which may characterise PpIX fluorescence and in turn improve knowledge of photosensitiser delivery [50]. A detailed description of this will be outlined in the forthcoming chapter.

2.2.3 Summary
The key features together with the advantages and disadvantages associated with each optical diagnostic technique for their potential use in PDT will be presented here. Table 2.1 lists and compares important characteristics of the above mentioned optical techniques. OCT and FI are non-contact techniques, which offer morphological information about tissue. OCT has a high resolution, however it has a limited penetration depth. FI provides biochemical information in addition to morphological, with a valuable overview of the tissue surface, but not at exact locations like fluorescence spectroscopy. Furthermore, FI can be technically intensive and difficult to use in the clinical setting. DRS is cheap to implement, has a very high elastic scattering signal intensity, although offers only morphological information relating to tissue characteristics. Raman spectroscopy provides extremely detailed molecular and biochemical tissue fingerprints, however, it is commonly employed in the NIR region as it is much weaker than the fluorescence signals in the UV-visible region. Determining the absorption and concentration of a photosensitiser via absorption spectroscopy is useful for PDT treatments. Nevertheless, sensitivity and tissue scattering somewhat negate this technique. LIFS is useful for biochemical and morphological information about tissue characteristics. The fluorescence signal can serve as a sensitive indicator of tissue biochemistry. LIFS and FI are both integral
parts of FD. Although FD can suffer from false positives, it has several notable strengths, such as; LIFS can be effectively implemented into the clinical environment and has the potential to offer an insight into fluorescence photobleaching during PDT, which has an appreciated role for monitoring the progression of treatment. Ultimately, LIFS may assist in optimising PDT treatment regimes. Therefore, the application of FD to PDT in dermatology will not go without a positive clinical impact.

Table 2.1 Strengths and weaknesses associated with some optical techniques employed for medical diagnostics.

<table>
<thead>
<tr>
<th>Optical Technique</th>
<th>Biochemical</th>
<th>Molecular</th>
<th>Morphological</th>
<th>Signal Strength</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT</td>
<td></td>
<td></td>
<td>✓</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>FI</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>DRS</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>+++</td>
<td>✓</td>
</tr>
<tr>
<td>RS</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>+</td>
<td>✓</td>
</tr>
<tr>
<td>AS</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>LIFS</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>++</td>
<td>✓</td>
</tr>
</tbody>
</table>

2.3 Optical Biopsy System (OBS)

The development of techniques such as those mentioned above is currently being explored in many centres to help with the diagnosis of different forms of cancer and other pathologies. The measurement and determination of fluorescence in patients is a challenging task, particularly in vivo. It is now recognised that early detection of cancer is key to improving survival and, as a result, the development of non-invasive in vivo diagnostic measures is important as a means to achieving this goal. From a clinical point of view, immediate diagnosis and earlier treatment of cancer would prove beneficial insofar as enhancing treatment outcomes. The optical biopsy system (OBS) operates on the principle of fluorescence spectroscopy, which offers a non-invasive in vivo method of detecting and monitoring in situ tissue fluorescence. Fluorescence diagnostic spectral signatures can be obtained in real time, which can assist clinicians during a treatment procedure.
2.3.1 Description of the Instrument
The OBS was designed and developed at the University of Glasgow, Glasgow, UK [51]. This point fluorescence spectroscopy system, depicted in Figure 2.2 incorporates a light emitting diode (LED) emitting at 400 nm, which corresponds to the maximum excitation wavelength (Soret band) of PpIX, optical fibres, fibre splitter, 455 nm long-pass filter, compact grating spectrometer, probe and a laptop computer. The output is coupled to a 400 µm core optical fibre probe. The probe – consisting of a single fibre – is placed in contact with the skin at the region of interest (ROI). This single fibre probe is used for both coupling the excitation light to the tissue site and collecting the emitted fluorescence signal, and is commonly used during fluorescence spectroscopy [52]. A fibre splitter enables the excitation light to be transported to the skin whilst ensuring that the fluorescence induced in the skin is recorded by a compact spectrometer. The USB2000 spectrometer is preceded by a 455 nm long-pass filter, which allows the backscattered excitation light to be removed. This specific spectrometer houses a Sony ILX511 2048-element linear silicon CCD array detector with wavelength units of 0.384 nm per pixel. Furthermore, the spectrometer, which quantifies the collected fluorescence signal is connected to a laptop computer and LabView programme software installed on the laptop displays the measured fluorescence spectra on the computer screen. The entire operation of the system is controlled using a laptop computer and custom written software. A schematic diagram of the system is illustrated in Figure 2.3. All components, except for the probe and laptop computer are housed in a box, which prevents the entry of stray light.
Figure 2.2 The Optical Biopsy System (OBS) used for fluorescence detection.

Figure 2.3 Schematic diagram of the OBS and the comprising internal components.
2.3.2 Operating the Optical Biopsy System

LabView was used for data acquisition and instrumental control. A Universal Serial Bus (USB) cable is connected from the spectrometer to the computer. The spectrometer was used to produce characteristic fluorescence spectral signals and thus measure the corresponding wavelengths and intensities. LabView programme software displayed the acquired fluorescence spectra on the laptop computer screen. The light exposure time is controlled by the operator via the LabView programme and varies the integration time from 500 ms to 2000 ms, depending on the desired signal to noise ratio (SNR). The probe was then placed, with a slight pressure in perpendicular contact with the ROI, such as a skin lesion to ensure consistency in the acquired fluorescence spectra.

![Graphs](image_url)

Figure 2.4 (a) LED light source excitation spectrum emitted from the OBS together with the absorption spectrum of PpIX; (b) Broad PpIX absorption spectrum together with characteristic PpIX fluorescence emission with a dominant peak at 635 nm.
The main purpose of the OBS was to perform noninvasive *in vivo* fluorescence measurements from patients presenting with various types of NMSC, such as sBCC and BD. Figure 2.4 (a) illustrates the LED spectra housed in the OBS and the five characteristic absorption peaks of the PpIX absorption spectrum. The LED excitation light source targets the highest absorption peak of PpIX, namely, the Soret band. Figure 2.4 (b) illustrates the typical PpIX fluorescence spectral signal observed and collected upon excitation with the LED.

### 2.4 Optical Biopsy System Reproducibility Technique

The induced fluorescence spectral signals are dependent on the sample composition. To ensure accurate measurements from patients, it was first necessary to investigate whether the system was capable of producing consistent and reproducible measurements in a laboratory setting under fixed conditions. The reproducibility technique designed for the OBS was similar to that routinely used when screening blood for porphyria – a condition that involves an abnormal sensitivity to light – and was performed using a Coproporphyrin Fluorescence Standard (CFS) [53]. Ghadially et al. [54] were the first to describe the red characteristic fluorescence and ascribed it to porphyrins. It was decided, therefore, to develop a new reproducibility technique based on the use of coproporphyrin. Ultimately, the goal here was to monitor the stability of the OBS.

#### 2.4.1 Laboratory Reproducibility Set-Up

Firstly, in a darkened room, the probe was surrounded by a black rubber cushion and a dark reference spectrum was recorded on the OBS. This corrected for stray light and fluorescence from the optical fibres and probe, and was taken before any fluorescence measurements were recorded. For all the reproducibility measurements, the integration time was set at 2000 ms, which provided optimum SNR. Once this was carried out the probe was positioned. Figure 2.5 (a) illustrates the laboratory experimental set-up, which consisted of a quartz cuvette housed in an opaque alignment holder, which tightly placed the fibre optic probe in perpendicular contact onto the cuvette. To reduce any interference from surrounding stray light the holder covered the cuvette on all four sides. Using a pipette, the cuvette was loaded with 2 ml of CFS and eight fluorescence spectral measurements were recorded at the fixed
position and displayed on the laptop computer screen. This number of measurements was chosen for the purposes of uncertainty. The fluorescence spectral measurements were performed in a darkened room to minimise artefacts from ambient room light. Finally, the spectral results were saved automatically as Microsoft® Excel spreadsheets, enabling data analysis to be performed at the user’s discretion.

Figure 2.5 (a) Quartz cuvette surrounded by an in-house designed cuvette holder, which enables the fibre optic probe to be placed in contact orthogonally to the front face of the cuvette; (b) characteristic red fluorescence exhibited from the CFS, owing to the porphyrin in the solution.

2.4.2 Coproporphyrin III Fluorescence Standard (CFS)

The stability of the OBS was monitored – as described above – against a known solution of CFS (10 ml ± 0.2 ml Coproporphyrin III in 1.0 N HCL, Porphyrin Products, Logan, Utah). This standard was used as it is stable for prolonged time periods – 1 year – instead of PpIX, which is stable for merely 24 hours and is highly photolabile. Using the spectrophotometer, the CFS is seen to emit peaks around 595 nm and 650 nm – Figure 2.6 – while PpIX has a large peak around 635 nm. Given its greater stability, CFS was an ideal candidate for ascertaining and comparing fluorescence spectral measurements.
Figure 2.6 Characteristic (left in black) absorption spectrum of the CFS; (right in red) fluorescence emission with peaks of the CFS at 595 nm and 650 nm recorded using a spectrophotometer (Hitachi Spectrophotometer U-3010).

2.4.3 Optical Biopsy System Reproducibility

Firstly, it was important to examine the output power of the OBS light source. This was determined by the experimental set-up as shown in Figure 2.7. The probe was placed in contact and fixed to the detector head of an Ophir 2000 power-meter. Readings were subsequently recorded hourly in a darkened room throughout the day. The output power exiting the distal end of the probe was consistently found to be 0.03 mW. The power from the OBS light source can adequately induce measurable fluorescence spectra at only a fraction of the treatment wavelength power resulting in a negligible degree of photobleaching from this system [51]. Photobleaching is examined further in Section 2.4.5. The fluorescence peaks around 595 nm and 650 nm were pertinent for monitoring the stability of the OBS. OBS reproducibility was checked periodically – approximately every 4 weeks during the collection of patient PpIX fluorescence – by acquiring and measuring CFS peaks. These fluorescence measurements were recorded as a function of wavelength allowing for both fluorescence intensity levels and wavelengths to be examined. These peaks were
informative in determining whether the fluorescence intensity levels or the wavelengths or both were drifting over time, highlighting any significant changes in the performance of the OBS. Therefore, by accounting for system drift, any undesired deviations in the measurements were established.

![Laboratory set-up for measuring the output power exiting the distal end of probe.](image)

**Figure 2.7** Laboratory set-up for measuring the output power exiting the distal end of probe.

### 2.4.4 Fluorescence Intensity and Wavelength Consistency

Maximum fluorescence intensity levels were ascertained from the CFS peaks. These levels and the change thereof were investigated hourly over the course of a day. Eight fluorescence spectral measurements were recorded using the OBS every hour for eight hours over the course of one day and the data were subsequently analysed. The mean ($\mu$) ± standard deviation ($\sigma$) and the coefficient of variation (COV) were recorded. The COV is expressed by the following equation

$$COV = \frac{\sigma}{\mu} \times 100\%$$  \hspace{1cm} (2.1)
This routine procedure was then repeated approximately four weeks later. The results were compared using \( \mu \), \( \sigma \) and the COV. This analysis ensured system consistency and reproducibility. The observed maximum fluorescence intensities were recorded as functions of wavelength. It was also important to investigate whether there was any drift associated with these wavelengths. Therefore, the wavelengths corresponding to the maximum fluorescence intensities were also logged. Similar to above, the wavelengths were analysed using \( \mu \), \( \sigma \) and the COV. The wavelengths of each of the two peaks depicted below in Figure 2.8 were measured by single-pixel sampling.

![Characteristic spectra recorded from the CFS solution exhibited around 595 nm and 650 nm using the OBS; (b) Mean and associated standard deviation corresponding to the fluorescence intensities and wavelengths of the CFS peaks recorded on eight different days.](image)

**Figure 2.8 (a)** Characteristic spectra recorded from the CFS solution exhibited around 595 nm and 650 nm using the OBS; (b) Mean and associated standard deviation corresponding to the fluorescence intensities and wavelengths of the CFS peaks recorded on eight different days.

The spectra depicted in Figure 2.8 (a) are representative of the fluctuations seen during the reproducibility process of the OBS. Reproducibility measurements were performed on eight different days and the corresponding fluorescence intensities are...
depicted. The overall mean of the eight spectral fluorescence measurements is highlighted by a thick solid line. The means and standard deviations for both the fluorescence intensity and wavelength values corresponding to the CFS are illustrated in Figure 2.8 (b). Tables 2.2 – 2.5 offer a more detailed description of how the data analysis of the peaks presented in Figure 2.8 (a) and (b) were performed. Further analysis was performed on these measurements, where mean fluorescence intensity values of 40.24 arb. units (Table 2.3) and 32.03 arb. units (Table 2.5) with associated standard deviation of 1.98 and 0.67, respectively were calculated. Moreover, the COV were recorded as < 5 %. This data analysis method was then repeated to investigate the consistency of the wavelengths where the fluorescence peaks feature. Mean wavelengths were seen at 596 nm and 652 nm with associated standard deviations of 0.74 and 0.93, respectively. The COV recorded in this instance were 0.10 % (596 nm) and 0.14 % (652 nm).

Table 2.2 Wavelength (595 nm) and fluorescence intensity consistency measurements recorded during one day. The values in red are indicative of the mean wavelength (nm) and mean fluorescence intensity (arb. units) for a series of eight measurements.
### Table 2.3 Wavelength (595 nm) and fluorescence intensity consistency measurements over 8 days. The values in red are indicative of the mean wavelength (nm) and mean fluorescence intensity (arb. units) for a series of eight measurements recorded over the course of one day as shown in previously in Table 2.2.

<table>
<thead>
<tr>
<th>Days 1-8</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>µ</th>
<th>σ</th>
<th>COV</th>
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<tbody>
<tr>
<td>Wavelength (nm)</td>
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<td>597.43</td>
<td>596.66</td>
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<td>595.89</td>
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<tr>
<td>Fluorescence Intensity (arb. units)</td>
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<td>38.27</td>
<td>43.10</td>
<td>39.58</td>
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<td>40.19</td>
<td>43.51</td>
<td>38.77</td>
<td>40.24</td>
<td>1.98</td>
<td>4.92%</td>
</tr>
</tbody>
</table>

### Table 2.4 Wavelength (650 nm) and fluorescence intensity consistency measurements recorded during one day.

<table>
<thead>
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<th>Day 1</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Hours</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>µ</td>
<td>σ</td>
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<tr>
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<td>652.72</td>
<td>650.80</td>
<td>651.19</td>
<td>651.95</td>
<td>652.72</td>
<td>651.57</td>
<td>652.72</td>
<td>651.76</td>
<td>0.92</td>
<td></td>
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<tr>
<td>Fluorescence Intensity (arb. units)</td>
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<td>31.19</td>
<td>31.58</td>
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<td>31.49</td>
<td>32.56</td>
<td>32.43</td>
<td>32.58</td>
<td>31.86</td>
<td>0.58</td>
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</table>

### Table 2.5 Wavelength (650 nm) and fluorescence intensity consistency measurements over 8 days.

<table>
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<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
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<th>8</th>
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<td>Wavelength (nm)</td>
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<td>653.30</td>
<td>652.53</td>
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<td>652.91</td>
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<tr>
<td>Fluorescence Intensity (arb. units)</td>
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<td>31.74</td>
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<td>31.37</td>
<td>31.79</td>
<td>33.25</td>
<td>31.37</td>
<td>32.03</td>
<td>0.67</td>
<td>2.09%</td>
</tr>
</tbody>
</table>
2.4.5 Photobleaching Effects of the Optical Biopsy System

Photobleaching occurs when fluorescence molecules are exposed to visible light. This raises the question as to whether the OBS might cause photobleaching during the time taken to acquire fluorescence spectra. To investigate the effect of photobleaching from the system, the CFS fluorescence peaks recorded over the course of a day were quantitatively assessed and compared. The overall mean of the fluorescence intensity peaks were 39.03 (596 nm) and 31.86 (651 nm) with associated standard deviations of 0.78 and 0.58, respectively. No systematic decrease in fluorescence intensity was observed from the first to the last of the eight measurements over the time course of the day. The values presented in table 2.3 and 2.5 emphasise insignificant photobleaching. Moreover, the wavelength region between 600 nm and 650 nm was examined as this is the region where the dominant 635 nm PpIX fluorescence peak exists. Therefore, fluorescence intensities recorded at wavelengths between those presented in table 2.2 and 2.4 were examined.

Each day eight measurements were taken hourly. The hourly mean was recorded for a total of eight hours, which determined the overall mean for that day. Each of the eight hourly means was divided by this overall mean, at each corresponding wavelength from 600 nm to 650 nm. This is plotted in figure 2.9. The overall mean was taken as 1 and the hourly means, each as a ratio of this. Therefore, each of the eight hourly means is a ratio relative to the overall mean at each corresponding wavelength. The small differences between the fluorescence intensity measurements as illustrated in figure 2.9 – 1 is indicative of the first measurement recorded at 9 am (first hourly mean) and 8 represents the last measurement at 4 pm (last hourly mean) – further supported the fact that photobleaching from this system was negligible. Thus, using CFS as a fluorescence standard solution, the OBS was proven to be valid as a fluorescence detection system.
Figure 2.9 Similar fluorescence intensities as a function of wavelength from eight different measurements. 1 represents the first measurement (first hourly mean), while 8 signifies the last measurement (last hourly mean). Each day eight measurements were taken hourly. The hourly mean was recorded for a total of eight hours, which determined the overall mean for that day. Each of the eight hourly means was divided by this overall mean, at each corresponding wavelength from 600 nm to 650 nm. The overall mean was taken as 1 and the hourly means, each as a ratio of this. Therefore, each of the eight hourly means is a ratio relative to the overall mean at each corresponding wavelength.

2.5 Patients in Photodynamic Therapy Clinics
Endogenous skin tissue fluorophores exhibit characteristic autofluorescence when illuminated with light of specific wavelengths [5]. However, tissue autofluorescence signals may be weak and difficult to interpret clinically [3]. In light of this, PpIX fluorescence analysis is usually performed, particularly for patients in clinics. The Woods lamp, illustrated in Figure 2.10 is a tool, which may be used routinely in PDT clinics to visually assess PpIX fluorescence and guide clinicians to optimum treatment sites. This light source emits between 375 – 440 nm and operates on the fact that
PpIX preferentially accumulates in lesional cells with much less accumulation in surrounding normal skin. The Woods lamp is used routinely in the Scottish PDT Centre at Ninewells Hospital. Immediately before a patient receives PDT, the lesion is subjected to Woods light exposure. This exposes the entire surface area of the lesion inducing fluorescence which in turn, allows for the accurate demarcation of exact tissue – tumour margins. Immediately after PDT treatment, the lesion is again investigated for remnants of PpIX fluorescence. As expected, after treatment, there is no remaining PpIX fluorescence visible to the naked eye. This suggests that all of the PpIX fluorescence has diminished due to photobleaching. However, it is possible only to observe the surface PpIX fluorescence with the naked eye, which lacks quantitative information. On the other hand, the OBS incorporating a fibre optic probe samples fluorescence from smaller surface areas with high sensitivity, while providing quantitative spectral information in the process [52].

Figure 2.10 Woods Lamp used routinely in a PDT clinic at Ninewells Hospital.
Figure 2.11 (a) A normal tissue site before – and after – incubation with ALA observed under normal room lighting; fluorescence (b) observed before incubation with ALA using the OBS; (c) after incubation with ALA observed under Woods lamp exposure; (d) after incubation with ALA using the OBS.
ALA was topically applied for 6 hours to a normal skin site on the right forearm. Under normal room lighting conditions, as shown in Figure 2.11 (a) fluorescence is not visible prior to and after ALA application, while (b) depicts autofluorescence recorded before ALA application. Moreover, Figure 2.11 (c) and (d) shows evidence of PpIX fluorescence after ALA application observed when using the Woods lamp and the OBS, respectively. The characteristic red coloured emission from the skin pertains to the area where ALA was administered and can be clearly distinguished from the surrounding skin, not incubated with ALA. This phenomenon is well established and consequently has been implemented as a routine procedure performed in PDT at some centres. The potential of using the OBS in clinics is highlighted by the quantitative characteristic spectral data provided by Figure 2.11 (d).

Tumour tissue has a higher metabolic rate than normal skin tissue and exhibits more intense PpIX fluorescence [55]. Figure 2.12 (a) depicts a sBCC after a 3 hour application of Metvix® and immediately pre-treatment, while (c) is representative of the same sBCC post-treatment under normal room lighting conditions. There are virtually no marked differences seen between the images. However, under Woods lamp exposure, the two corresponding images can be distinguished on the merit of PpIX fluorescence. As expected, Figure 2.12 (b) recorded pre-PDT exhibits PPIX fluorescence, while (d) displays no PPIX fluorescence, relating to post PDT. This is the central topic of the research discussed in the forthcoming chapter.

Visual inspection of PpIX fluorescence using the Wood’s lamp is beneficial but it is difficult to quantitify the fluorescence. As shown by Figure 2.11, the OBS has the advantage of acquiring quantitative information relating to PpIX fluorescence. Point measurements record an entire fluorescence spectrum for a given wavelength and can be repeated at different sites where the photosensitiser pro-drug was applied. It is limited by the fact that it only provides data at the point of measurement and, for this reason, readings are taken at several locations.
Figure 2.12 Images recorded pre-PDT treatment; (a) under normal room lighting; (b) under Woods lamp exposure; Images recorded post-PDT treatment; (c) under normal room lighting; and (d) under Woods lamp exposure.
2.6 Optical Biopsy System in Clinical Practice

The reproducibility results presented in Table 2.2 to 2.5 further supports the fact that the performance of this point fluorescence spectroscopy was a reliable system for detecting fluorescence. The OBS provides characteristic fluorescence spectra from lesions that relate to the disease status of skin tissue, therefore fluorescence can be used diagnostically, such as for superficial NMSC. While the CFS has fluorescence peaks at 595 nm and 650 nm, PpIX fluorescence has a large peak at 635 nm and a smaller peak at 705 nm. It is these latter peaks, which are important in PD in PDT. To induce adequate PpIX fluorescence intensities levels from skin tissue (normal or lesion), it is first necessary to administer a therapeutic photosensitiser pro-drug. Various pro-drugs, which are topically applied – in the form of creams – to skin lesions, may be used in porphyrin based topical PDT treatment regimes of NMSC. The kinetics of PpIX fluorescence may be assessed quantitatively during PDT treatments using the OBS. Therefore, this assessment has the potential to monitor PpIX photobleaching, which is linked to the production of singlet oxygen [56]. This, in turn, may provide an indication of the PDD administered during PDT treatment [57]. It follows that information pertaining to in vivo PpIX fluorescence may assist in the optimisation of future PDT treatment regimes. In light of this, the OBS offers an extra dimension to the localisation of tumour fluorescence. Monitoring PpIX fluorescence kinetics may be used to retrospectively highlight treatment failures and further improve knowledge of photosensitiser delivery by assisting in the choice of optimal photosensitiser pro-drug for PDT [50]. Furthermore, using the OBS, PpIX fluorescence could be analysed and examined as a function of time, where the pro-drug could be applied for varying time courses. Autofluorescence can also be investigated. However, the focus of the research presented here is centred on the well defined PpIX fluorescence emission, which is usually more intense and selective. These are among some of the potential uses of the OBS in PDT clinics.

The diagnostic and monitoring power of PpIX fluorescence has been extended to other organs, for example, the brain. The detection in PpIX fluorescence has been used in neurosurgery at Ninewells Hospital. As standard procedure, tumour resection is performed by the surgeon. However, it is most helpful for surgeons to have guidance to objectively discriminate between normal and cancerous tissue, and to ensure accurate resection. PpIX fluorescence detected from brain tissue can be
assessed by the OBS and has been subsequently used to assist in the surgical resection of brain tumour tissue.

2.7 Conclusion

In this chapter, a useful synopsis of potential techniques for medical diagnosis, particularly in the context of PDT has been presented. Each technique has strengths and weaknesses; ranging from the sharp, narrow and molecular specific Raman spectra obtained with expensive and relatively complex instrumentation to strong but broad scattering and fluorescence signals collected with comparatively cheaper equipment. Optical diagnosis offers non-invasive, in situ and real time examination of tissue pathology. Rather than clinicians randomly sampling sites for invasive excisional biopsies, optical biopsy can potentially guide and reduce targeting biopsy collection and define tumour margins [29]. Ultimately, from non-invasive optical measurements of tissues, the goal is to provide quantitative biochemical, molecular and morphological information with high sensitivity and specificity for tissue characterisation relating to the diseased state of tissue. Biochemical changes in tissue precede morphological changes and this is an important issue when considering techniques for the early diagnosis of pre-cancer and cancer [58]. The reproducibility of a fluorescence spectroscopy system, namely the OBS was ascertained. The reproducibility technique described here was adopted from that used when screening blood for porphyria. The system demonstrated a high level of consistency throughout the reproducibility process, providing a low COV of 0.10 % and 0.14 % associated with the wavelengths, 595 nm and 650 nm while 4.92 % (595 nm) and 2.09 % (650 nm) was associated with the fluorescence intensity deviations. These results confirmed the potential of using this system in a clinical setting, with a view to detecting and monitoring PpIX fluorescence from patients presenting with NMSC. It is of clinical importance to be able to determine accurate PpIX fluorescence signals in vivo from patients and the change thereof, without any interference from an unreliable fluorescence detection system. The OBS offers a quantifiable measure of peak PpIX fluorescence when compared to the conventional Woods lamp. Optical diagnostic techniques for in vivo clinical applications are ever increasing and perhaps the evolution of optical spectroscopy aids widespread clinical acceptance. Fluorescence spectroscopy is used to quantify biomarkers in vivo for the early diagnosis and
successful treatment of NMSCs. Fluorescence biomarkers can be used concomitantly for PD and PDT treatment. Photosensitiser pro-drugs are an integral part of PDT. Knowledge of photosensitiser uptake and photobleaching are essential to PDT dosimetry [47-50,56,57]. Ultimately, fluorescence spectroscopy appears to be the modality of choice for PD in PDT as it can be employed to optimise and monitor treatments.
2.8 References


Chapter 2 – Photodiagnosis in Topical Photodynamic Therapy


Chapter 3 – Topical Photodynamic Therapy in Dermatology

3.1 Introduction

A pragmatic application of fluorescence spectroscopy to routine clinical PDT will be presented in this chapter. The characteristics of PpIX fluorescence in sBCC and BD following application of ALA and its methyl ester, MAL before, during and after PDT were investigated in 40 patients. Photosensitiser pro-drug penetration can limit PDT efficacy and understanding the characteristics of PpIX fluorescence through fluorescence spectroscopy may improve knowledge of photosensitiser delivery. Fluorescence intensity was assessed quantitatively, and the rate of photobleaching was determined by fitting an exponential decay. As a secondary end-point, PDT-induced pain, was also measured continuously during treatment using a novel hand-held device, known as a pain logger.

Although, – as mentioned earlier – there are guidelines for the use of PDT [1,2], variables in treatment regimes exist, due to there being no clear evidence regarding optimal treatment parameters [3]. Pain can be problematic during irradiation and factors influencing this are not well defined. MAL is the only licensed formulation for topical PDT. Due to its lipophilicity, MAL should theoretically be more specific and penetrate deeper into tumour tissue than ALA [4]. However, there is a relative paucity of comparative studies of efficacy and no convincing data to support this [5,6].

As mentioned previously in Chapter 2, Section 2.2.2.4, LIFS is a valuable optical technique that may be used clinically as a tool for early diagnosis of tumours and facilitate monitoring of PDT [7]. Optimal PDT regimes would provide minimal damage to normal skin while delivering an effective therapeutic result to diseased skin. The selection of the light dose delivered to the tumour is important for successful PDT. Knowledge of the characteristics of ALA- and MAL-induced PPIX fluorescence and photobleaching during PDT may, therefore, help achieve optimisation of PDT regimes. The purpose of the work discussed here was to investigate and compare ALA and MAL-induced PPIX fluorescence in vivo in patients with BD and sBCC receiving topical PDT. The OBS as previously described in Chapter 2, Section 2.3, was used. In

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addition, a secondary aim was to use a novel hand-held device known as a pain logger, to record the characteristics of the pain experienced during PDT.

3.2 Materials and Methods
3.2.1 Patients
Fluorescence readings and pain assessments, as detailed below, were performed in forty patients (23 F:17 M mean age 72 years) with clinically and/or histologically diagnosed BD (n=20) and sBCC (n=20) referred for routine PDT in the Photobiology Unit, Ninewells Hospital and Medical School, Dundee. Fluorescence and pain are assessed as part of our normal clinical practice, so this was not considered to be a departure from routine patient care. Data were collected from one untreated lesion at any body site.

3.2.2 Cream Application
After routine surface preparation, ALA (5-ALA, 20% (w/w) in oil in water base, Manmed Pharmaceuticals, UK) was applied to lesions of BD (n=10) and sBCC (n=10) for 4 or 6 hours, respectively. MAL cream (Mevix™, 160 mg/g (16%) methyl aminolevulinate hydrochloride, Galderma, UK) was applied to the remaining lesions; BD (n=10) and sBCC (n=10) for 3 hours. Lesions were occluded with Tegaderm™ and Mepore™ dressings during the incubation periods prior to removal and any excess cream was then gently wiped off.

![Common photosensitiser pro-drugs employed in PDT (a) MAL ; and (b) ALA.](image)

**Figure 3.1** Common photosensitiser pro-drugs employed in PDT (a) MAL ; and (b) ALA.
3.2.3 Photodynamic Therapy
The two LED light sources used for the PDT treatments were the Aktilite CL16 and CL128 (Photocure ASA, Hoffsveien 48, N-0377 Oslo, Norway). These commercial devices have identical spectra, where the peak irradiance occurs at a wavelength of 632 nm with a full width half maximum (FWHM) of 19 nm. Both light sources were calibrated and traceable to national measurement standards [8]. A total light dose of 75 J/cm$^2$ was used during PDT at a distance of 5 – 8 cm from the skin surface. The total treatment times varied between 13-18 minutes, (mean = 943 seconds) depending on the area being treated and the skin surface irradiance. Typical skin surface irradiances ranged from 70 to 90 mW/cm$^2$ (mean = 80 mW/cm$^2$).

3.2.4 Fluorescence Spectroscopy
Each lesion was examined by fluorescence spectroscopy during PDT. Fluorescence spectra were acquired in this study using a custom built optical biopsy system (OBS) (University of Glasgow, Glasgow, UK), which was described in detail in Chapter 2, Section 2.3.1.

3.2.5 Fluorescence Detection and Photobleaching
In vivo PpIX fluorescence was induced in the skin and spectra were recorded by placing the probe perpendicularly in contact with the lesion. PpIX fluorescence emission spectra were recorded in vivo from all lesions at four different time points: prior to cream application (i.e., baseline fluorescence measurements), immediately before, mid-irradiation and immediately after PDT. Furthermore, additional fluorescence spectra were recorded as controls from normal skin located approximately 5 cm distal to the lesion. Treatment was interrupted briefly to allow for the mid-irradiation fluorescence measurements to be carried out. All measurements at each site were performed eight times and the mean of the eight readings was determined. For the purposes of localising the fluorescence measurements relative to the lesion, each lesion was visually separated into an outer region and an inner region. Subsequently, four measurements were taken – clockwise – from the outer region at 12, 3, 6 and 9. A further four measurements were taken from the inner region of the lesion, again at 12, 3, 6 and 9. Each measurement was performed in a darkened room to minimise artefacts from ambient room light. Moreover, the optical fibre probe was repositioned for each
individual measurement and each individual spectrum was recorded onto the computer. The exposure time of the excitation light used for acquiring the fluorescence spectra was 500 ms. It was assumed that during fluorescence detection the amount of photobleaching would be negligible owing to the low intensity and short exposure time of the excitation light. Peak PpIX fluorescence intensity was examined in each lesion at each time point. The peak emission wavelength at around 635 nm was used to quantify PpIX fluorescence. The baseline fluorescence spectra recorded prior to the application of the cream were subtracted from their corresponding fluorescence spectra measurements recorded at the three other time points. Thus, measurement of these PpIX fluorescence spectral data enabled the in vivo monitoring of PpIX photobleaching and fluorescence kinetics during topical PDT.

3.2.6 Pain

Patients are routinely asked to recall – after treatment – the maximum pain they experienced. For the purpose of pain assessments here, a novel pain logger device – Figure 3.2 – was implemented to assess PDT-induced pain. This hand-held device was the product of an in-house design, assembled at Ninewells Hospital and Medical School, Dundee, UK. The patient’s pain score is recorded continuously by the device according to the movement of a dial switch. The pain score could be set anywhere between 0 ( = no pain) and 10 ( = worst pain imaginable) and was under the control of the patient throughout the course of the treatment. After the treatment, the device is plugged into a computer via an USB connection. Subsequently, pain scores recorded over the entire treatment are retrieved using custom written software installed on the computer. Instead of recording a retrospective snapshot of pain experienced, as with a conventional visual analogue scale (VAS), this method allows an assessment of the time-course of pain throughout PDT. The patient is asked to move the dial in a clockwise or counter-clockwise direction on the device dependent on the degree of pain they are experiencing at that point in time.

Pain scores were recorded and using the area under the curve (AUC) technique, mean pain scores (MPS) were calculated for each patient and each group. These were determined according to the following equation:
Subsequently, the MPS for each group were compared. Using measurements from the pain logger, it was possible to establish the mean time, $\bar{t}$, when the pain was experienced during treatment. This was calculated using the following equation:

$$\bar{t} = \frac{\sum_{i=1}^{1200} p_i t_i}{\sum p_i}$$  \hspace{1cm} (3.2)$$

where $t_i$ is the time from the start of treatment to the point of measurement and $p_i$ is the pain experienced at a given time point of measurement.

Figure 3.2 Pain experienced by patients during PDT was continuously assessed by the novel pain logger device.
3.2.7 Statistical Analysis
In all analyses of PPIX fluorescence and pain data, values are given as mean ± standard deviation ($\sigma$). Comparisons between the means of the PpIX fluorescence and pain data acquired from each of the four groups were performed using the independent Student’s $t$-test analysis. Statistical significance was taken as $P < 0.05$.

3.3 Results
3.3.1 In vivo PpIX Fluorescence and Photobleaching
Eight in vivo PpIX fluorescence spectra were recorded from each of the ten patients, at the four different time points in each of the four groups:

1. **Group 1** (n=10) patients with sBCC treated with ALA-PDT
2. **Group 2** (n=10) patients with sBCC treated with MAL-PDT
3. **Group 3** (n=10) patients with BD treated with ALA-PDT
4. **Group 4** (n=10) patients with BD treated with MAL-PDT

The significance of PD in PDT is well known and was described previously in Chapter 2. Figure 3.3 shows typical PpIX fluorescence detected from a skin lesion, in this case sBCC after 6 h of ALA application. The PpIX fluorescence observed from the lesion is characteristically increased relative to the surrounding normal skin tissue also exposed to ALA. Furthermore, autofluorescence from the lesion is greatly reduced when compared with the surrounding normal skin [9]. The green line is indicative of normal skin that was not incubated with ALA and here the PpIX fluorescence intensity is so low that it may be considered as background noise.
Figure 3.3 Fluorescence intensity spectra illustrating the differences in PpIX fluorescence, from a sBCC lesion (-) and surrounding normal skin tissue (−) 6 h after ALA application. Normal skin tissue that has not been incubated with ALA is also shown (−). Reduced autofluorescence from the lesion (−) is typical when compared to the surrounding normal skin tissue.

Figure 3.4 (a), (b), (c) and (d) illustrates mean PpIX fluorescence intensity spectra from 10 patients in each of the four groups 1, 2, 3 and 4, respectively, at the three time points of treatment. The maximum PPIX fluorescence intensity recorded immediately before PDT ( = Maximum) for group 1, 2, 3 and 4 is equal to 71, 50, 39 and 33 arbitrary units, respectively. The PpIX fluorescence intensities recorded at the mid-irradiation and immediately after PDT time points for all groups are expressed as percentages of their corresponding maxima and are seen to be less than 10 % and 5 %, respectively.

The mean PPIX fluorescence intensity was shown to decrease from immediately before to immediately after PDT, illustrating how peak PpIX fluorescence reduces as treatment progresses and photobleaching occurs. The results presented in Figure 3.4 demonstrate in vivo photobleaching of PpIX during topical PDT.
Figure 3.4 Mean PpIX fluorescence intensity spectra from patients presenting with (a) sBCC after ALA application (6 h) (n=10); (b) sBCC after MAL application (3 h) (n=10); (c) BD after ALA application (4 h) (n=10); and (d) BD after MAL application (3 h) (n=10), recorded immediately before PDT (-), mid-irradiation (-) and immediately post-PDT (-).
Photobleaching was assumed to follow a single exponential decay curve. A photobleaching time constant, $\tau_{PB}$, corresponds to a $\frac{1}{e}$ (~37%) reduction in PPIX fluorescence intensity. $\tau_{PB}$ of 170 s for group 1 represents the best fit to the data presented in Figure 3.5 (a).

Figure 3.5 Reduction in mean normalised PpIX fluorescence intensity during PDT recorded from patients presenting with (a) sBCC after ALA application (6 h) (n=10); (b) sBCC after MAL application (3 h) (n=10); (c) BD after ALA application (4 h) (n=10); and (d) BD after MAL application (3 h) (n=10), allows for the monitoring of in vivo photobleaching. Diamonds (◊) are representative of data from individual lesions. A best-fit exponential is shown, and the time for fluorescence reduction to 50% is indicated.
Photobleaching time constants, $\tau_{PB}^2 = 165$ s, $\tau_{PB}^3 = 165$ s and $\tau_{PB}^4 = 176$ s, were also determined for the remaining groups 2 (Figure 3.5 (b)), 3 (Figure 3.5 (c)) and 4 (Figure 3.5 (d)), respectively. These time constants are in close agreement with each other. Furthermore, the fluorescence half-life, which is the time for fluorescence to decrease to 50% of the normalised PpIX fluorescence intensity, could be determined for group 1 from Figure 3.4 (a) and was found to be 117 s (approximately 2 minutes). Similar fluorescence half-lives of 112 s, 113 s, and 120 s were recorded for group 2 (Figure 3.5 (b)), 3 (Figure 3.5 (c)) and 4 (Figure 3.5 (d)), respectively.

3.3.2 A Comparison between PpIX Fluorescence Induced by either ALA or MAL

A comparison between ALA- and MAL-induced PpIX fluorescence characteristics was also undertaken. Figure 3.6 (a) shows the comparison between group 1 and group 2. An apparently higher peak PpIX fluorescence intensity was seen following ALA application compared to MAL, although this was not significant ($n = 20$ ; $p = 0.116$). Indeed, there was also no significant difference between ALA and MAL-induced PpIX intensities at the other three time points. ($n = 20$; $p = 0.737$, $p = 0.078$, $p = 0.871$). Figure 3.6 (b) compares group 3 to group 4. Again, there was an apparently higher peak PpIX fluorescence intensity immediately before PDT following ALA application but this was not significantly different from the data obtained after MAL application at this or any of the other time points. ($n=20$; $p = 0.389$, $p = 0.462$, $p = 0.772$, $p = 0.470$).

3.3.3 A Comparison between PpIX Fluorescence Induced in sBCC and BD

Lesion types were compared with each other after application of the same cream. PpIX fluorescence intensity appeared higher in sBCC than BD immediately before PDT. Figure 3.6 (c) represents the comparison of group 2 to group 4. No significant difference was found in the peak PpIX fluorescence intensities induced after MAL application between sBCC and BD at any of the time points ($n=20$; $p = 0.807$, $p = 0.076$, $p = 0.893$, $p = 0.225$). Group 1 was compared to group 3 as illustrated in Figure 3.6 (d). No significant difference was found in PpIX fluorescence between BCC and BD before ALA application or after PDT ($n = 20$; $p = 0.745$, $p = 0.116$). However, PpIX fluorescence was significantly higher in sBCC than BD after ALA application immediately before and mid-PDT ($n=20$; $p = 0.016$, $p = 0.049$).
3.3.4 Pain Experienced During ALA-PDT and MAL-PDT

To obtain a more detailed analysis of pain experienced throughout the irradiation of PDT we used a novel hand-held pain logger device, which documented the time course of pain experienced. Figure 3.7 illustrates different time courses of pain experienced by two patients both presenting with sBCC but receiving either ALA-PDT or MAL-PDT and two patients both presenting with BD but receiving either ALA-PDT or MAL-PDT. For example, one patient from group 1 felt an intense pain almost immediately after irradiation commenced. Subsequently, there was a slight reduction
in pain, which then gradually decreased in steps as the treatment progressed. After 400 s (approximately 7 minutes), the pain intensity experienced by the patient was very low and remained so for the remainder of the treatment. In contrast to this, another patient from group 2 illustrated a cyclical pattern of pain experienced. The pain intensity fluctuated up and down during the entire treatment, indicating that the patient was subjected to sharper bursts of pain at different times during PDT. During these different time courses, the pain score could be assessed in second increments throughout PDT.

![Figure 3.7](image)

**Figure 3.7** Different time courses of pain experienced by (a) two patients both presenting with sBCC but receiving either ALA-PDT or MAL-PDT; and (b) two patients both presenting with BD but receiving either ALA-PDT or MAL-PDT.

Figure 3.8 demonstrates the MPS ± σ for each group. When comparing mean MPS values between ALA-PDT and MAL-PDT in lesions of sBCC, (group 1 = 2.79 ± 2.50 - vs- group 2 = 0.88 ± 1.28; n = 19; p = 0.055) and BD, (group 3 = 2.91 ± 2.79 -vs- group 4 = 3.70 ± 2.61; n = 20; p = 0.522) no significance difference was found between
groups. For ALA-PDT, lesions of sBCC and BD (group 1 -vs- group 3) were compared to each other and no significance difference in mean MPS was found between lesion types \((n = 20; p = 0.920)\). However, lesions of sBCC and BD treated with MAL-PDT (group 2 -vs- group 4) were compared and mean MPS values were significantly higher for BD than sBCC. \((n = 19; p = 0.009)\).

**Figure 3.8** The mean pain score (MPS) ± σ for each group recorded using the pain logger.
Figure 3.9 illustrates the MPS for the men and women who participated in this study. No significance difference was found between the gender types. (n = 39; p = 0.062).

Figure 3.9 The effect of gender on the mean pain score (MPS) assessed using the pain logger. The pain level is represented by the MPS with the error bars representing the standard deviation (\( \sigma \)) associated with the MPS.
It was also of interest to establish the mean time, $\bar{t}$, when the pain was experienced during PDT for each group. Figure 3.10 demonstrates that the majority of the pain was experienced in the second quarter of the treatment period, ranging from 357 s to 549 s (approximately 6 minutes to 9 minutes).

**Figure 3.10** The majority of the pain experienced over the PDT treatment period for each group is shown to be experienced in the first half of the treatment period. Horizontal lines represent 25, 50, 75 and 100 % of the irradiation period (seconds). The mean time, $\bar{t}$, is represented by the 4 data points above (■,●,▲,▼) with the error bars representing the standard deviation associated with the mean time, $\bar{t}$.
3.4 Discussion

3.4.1 PpIX Fluorescence and Photobleaching

In the work reported here, we investigated the effect of ALA and MAL application on PpIX fluorescence when treating sBCC and BD with topical PDT in 40 patients. Our results suggest that the PpIX fluorescence intensities measured immediately before-, mid- and immediately after- ALA-PDT and MAL-PDT are not significantly different and are in close agreement with each other. Immediately after PDT, the maximum PpIX fluorescence intensity was reduced, in all groups, to less than 5 % of that recorded immediately before PDT. This would seem to indicate that the prodrugs have played their role during PDT where the PpIX fluorescence has become photochemically destroyed, i.e., photobleached [10]. However, we need to be cautious in this interpretation as de Haas et al. [11] pointed out that large spatial variations in the distribution of PpIX fluorescence, especially at depth, may limit the detection of PpIX fluorescence emanating from deeper areas of sBCC using optical diagnosis [12].

The fact that the surface PpIX fluorescence intensities detected mid- and immediately after-PDT are very similar is of interest. According to Hewitt et al. [13] they found PpIX fluorescence to be reduced markedly after only 100 s (dose = 12 J/cm²) and Ericson et al. [14] reported a high rate of photobleaching at the beginning of treatment up to a cumulative light dose of 10 J/cm². We have shown that in vivo photobleaching in patients fits a single exponential decay curve, with similar time constants, $\tau_{PB}$, in all of the four groups. The time constant, $\tau_{PB}^{-1}$ of 170 s, obtained from Figure 3.4 (a) corresponds to a $\frac{1}{e}$ (~ 37 %) reduction in PpIX fluorescence intensity. Furthermore, the fluorescence half-life, which is 50 % of the PpIX fluorescence intensity could also be determined and was found to be 117 s. After 400 s (approximately 7 minutes), there was no further significant reduction seen in the PpIX fluorescence intensity.

However, Golub et al. [15] previously concluded that surface fluorescence spectroscopy does not allow the exact location of origin of fluorescence to be resolved. Therefore, it may be worthwhile, considering the use of longer wavelengths, such as 633 nm, that penetrate deeper into tissues and may assist in the determination of PpIX fluorescence from deeper within the lesion.

The maximum mean PpIX fluorescence intensity recorded with ALA
immediately before treatment appeared greater than with MAL but this was not found to be significant in this study. Higher PpIX fluorescence intensities were observed in sBCC than BD following ALA application and this likely reflects the longer duration of application of ALA for sBCC (6 h) compared with BD (4 h). In support of this, no significant difference was seen between MAL-induced PpIX fluorescence in sBCC and BD where the application period was 3 h for both.

3.4.2 Photodynamic Therapy-Induced Pain

Pain in PDT treatments is unpredictable and undeniably difficult to study, given there are many factors contributing to its intricate and complex nature. It can be a limiting factor to successful PDT [16]. During treatment, patients experience varying degrees of pain by what they describe as stinging, burning and prickling sensations. Using our pain logger, we attempted to examine this pain in more detail.

Conventionally, the patient is asked to indicate on a VAS (following completion of the treatment) the peak pain intensity level experienced during the light illumination period. A significant drawback to this method is that the patient retrospectively provides a mere snapshot of the pain experienced during their treatment. This is not necessarily a reliable representation of the pain experienced by the patient over the entire treatment period. The pain data acquired from the pain logger are advantageous and add another dimension to the VAS technique as they provide a second-by-second representation of the pain experienced by a patient over the complete treatment period. It has been suggested that MAL-PDT induces less pain than ALA-PDT in AK [17, 18] although it has been reported that there is no difference in the pain associated with either MAL-PDT or ALA-PDT in BCC [5, 19]. Our data support these latter observations, as we did not find a significant difference between the pain of MAL-PDT and ALA-PDT when used for BD and sBCC. Indeed, some patients reported higher pain scores with MAL-PDT than ALA-PDT. Interestingly, although we found significantly higher pain scores experienced by patients with BD treated with MAL-PDT compared with those with sBCC (Figure 3.8), no significant difference in peak PpIX fluorescence intensities was found at any time points with MAL-PDT for either BD or sBCC (Figure 3.6(c)), suggesting that factors other than fluorescence, intrinsic to the disease process itself may be important in determination of pain experienced. Pain intensity may be significantly different between lesion types.
and body sites and it has been reported that men find PDT more painful than women [16]. However, we did not find a gender effect; the difference in mean pain experienced by men (n = 16, 1 missing) and women (n = 23) was not found to be statistically significant (Figure 3.9). The mean time when the pain was experienced during treatment was in the second quarter of irradiation for both BD and sBCC and ALA and MAL PDT, i.e., in the first half of PDT (Figure 3.10). Although, PDT-induced pain is generally well tolerated, a significant proportion experience severe pain [3]. There is evidence to suggest that delivery of light during PDT using a lower irradiance is associated with less pain [20]. In an effort to overcome pain, recent research has led to the development of ambulatory PDT, which has shown very low pain scores when compared with conventional PDT [21,22]. Our data show that there tends to be more pain associated with the first half compared to the second half of treatment. Some patients experience variable intensities of pain throughout treatment while others find high pain levels initially which decrease during treatment.

3.5 Conclusion

In vivo PPIX fluorescence characteristics were monitored during PDT by fluorescence spectroscopy. The presented data demonstrate that this method of fluorescence monitoring can be used to investigate optimal treatment parameters for topical PDT. In vivo PpIX fluorescence was shown to decrease during irradiation, allowing the in vivo photobleaching of PpIX to be monitored. The PpIX fluorescence and photobleaching results recorded from 40 patients showed no significant differences between ALA- or MAL-induced PpIX fluorescence in lesions of sBCC and BD (P > 0.05) at all time points examined, indicating no detectable difference in PpIX kinetics for the two pro-drugs as assessed by these measures. We have therefore highlighted a consistent lack of significant difference in PPIX fluorescence kinetics when either ALA or MAL was topically applied to lesions. Furthermore, pain, as assessed by the logger device, showed high inter-individual variability and pain levels tended to be higher initially, decreasing during treatment. The pain data indicate no significant difference in pain associated with either ALA- or MAL-PDT in the patients studied (P > 0.05). Pain is of course very subjective and individual pain perception may be more of an issue than the photosensitiser pro-drug used. We have shown no clear advantage of either ALA or MAL based on the fluorescence and pain
data presented and on this basis alone both may be equally suitable for topical PDT. However, MAL is the only pro-drug licensed for use in topical PDT and although more expensive, is associated with a lower duration of application than ALA, resulting in shorter treatment times, which are clearly beneficial for patients.
3.6 References


Chapter 3 – Topical Photodynamic Therapy in Dermatology


18. Moloney, F.J. and P. Collins, “Randomized, double-blind, prospective study to


Chapter 4 – Monte Carlo Radiation Transfer Modelling

4.1 Introduction
Optimising clinical PDT treatment regimes requires studies involving theoretical radiation transfer simulations. MCRT methods may be used to compute the light dose within tissue and assist in accurate light dosimetry for PDT treatment planning [1]. MCRT modelling is the most flexible approach to describing photon transport in biological tissue. However, any model must first be validated by experimental and theoretical comparison. In this chapter, the principles upon which the MCRT method operates will be explained followed by a description of the MCRT model used throughout this research. A light scattering experiment carried out for the purpose of experimentally validating the model will be described. In these experiments, lasers were directed through a cuvette loaded with varying concentrations of a scattering agent, known as Intralipid 20 %. Three lasers emitting at different wavelengths (405 nm, 532 nm and 632 nm) were used to investigate the scattering of each laser beam by the Intralipid 20 % and consequently the distribution of the light as it propagated through the scattering agent. Intralipid 20 % was used as it has been shown to be representative of the light scattering characteristics associated with tissue [2].

In addition, contours of fluence rate simulated by other MCRT models – previously published in the literature – were reproduced by our MCRT model for further validation. Firstly, the distribution of 476 nm light in human aorta with collimated incident beams of varying diameters simulated by Keijzer et al., [3] were compared to the light distributions simulated by the MCRT model. Secondly, Jacques et al., [4] generated contours representing the PDD administered to a tumour over a simulated treatment time and these were compared with additional MCRT simulations.

4.2 Operating Principles of the Monte Carlo Radiation Transfer Method
The MCRT method was first put forward by Metropolis and Ulam in order to study physical processes using a statistical approach [5] and was later applied to the problem of light propagation in tissue by Wilson and Adam [6]. MCRT modelling is a technique that solves the transfer equation using the probabilistic nature of photon
interactions and has been used to simulate many such interactions, which have previously been modelled by several approximations, including the Kubelka-Munk theory [7, 8] and the diffusion approximation [9]. However, these techniques fail to accurately approximate light propagation at tissue surfaces, due to assumptions of isotropic scattering [10]. Conversely, MCRT modelling may be efficiently implemented for complicated tissue geometries and without restrictions in optical properties [11]. Furthermore, the MCRT technique provides accurate results for highly absorbing and scattering media at positions close to the surface and can handle the highly forward directed light scattering characteristics of tissue, i.e., anisotropic scattering [3, 12]. Wang et al. [13] developed a, now extensively used, MCRT model of light transport in multi-layered tissue for diagnostic and therapeutic applications of lasers in medicine. They modelled the tissue on a two dimensional (2D) grid system.

As previously shown in Chapter 1, Section 1.4, Equation 1.4, denotes the amount of radiant energy $dE_v$ transported across $dA$ within a $d\Omega$ over $dv$ during $dt$ in units Joules, $J$. This total energy may be equally split among MCRT photons, which are essentially luminosity – energy – packets. Each energy packet, $E_i$, is related to the specific intensity, $I_v$, by the following equation

$$I_v(r, \hat{s}, t) = \frac{\Delta E_i}{\cos \theta \Delta \lambda \Delta \Omega \Delta v \Delta t}$$

(4.1)

An energy packet, $E_i$, may be expressed in units Joules, $J$, as

$$\Delta E_i = \frac{L\Delta t}{N}$$

(4.2)

where $L$ is the luminosity – energy per second – in Watts, $\Delta t$ is the time in seconds and $N$ is the number of MCRT photons.

MCRT photon energy packets are related to actual numbers of real photons, $N_r$, through the following equation
\[ N_y = \frac{E_i}{h\nu_i} \]  \hspace{1cm} (4.3)

where \( h \) is Plank’s constant and \( \nu_i \) is the frequency of the MCRT photon energy packet.

The MCRT method is stochastic and statistical in nature, which describes the propagation of light by utilising probability distribution functions (PDFs). By sampling randomly from PDFs using cumulative distribution functions (CDFs), variables such as optical depths and photon scattering directions may be randomly chosen at interaction sites, enabling the position, direction and path of a photon to be determined. A computer generated pseudo-random number is used to represent a variable that is to be determined. The “random walk” of light photons may be determined as they traverse a tissue, until eventually getting absorbed or escape after traversing various distances. Consequently, photon path lengths between interactions are simulated. Variables are sampled randomly from the probability distribution function \( P(x) \), which defines the distribution of the variable \( x \) over the interval \((a \leq x \leq b)\) as shown below

\[ \int_a^b P(x)dx = 1 \]  \hspace{1cm} (4.4)

A value of \( x_i \), where \( i = 1,2,3,\ldots,n \) is randomly generated many times based on a pseudo-random generator, which generates a random number, \( \xi_i \),

\[ \int_a^{x_i} P(x)dx = \xi_i \]  \hspace{1cm} (4.5)

where \( \xi_i \) is a random number uniformly distributed over the interval \([0,1]\).
These equations are at the core of the MCRT method, which relies on random numbers. By implementing these equations it is possible to randomly sample the optical depth, $\tau$, the albedo, $a$, and the scattering angles; cosine of the deflection angle, $\cos \theta$ and the azimuthal angle, $\phi$, which are established by random number generators. A photon travels a physical distance, $S$, before an interaction occurs and this is given by the optical depth, $\tau$. The probability of travelling $\tau$ before an interaction is $e^{-\tau}$. To randomly sample an optical depth, $\tau$, the following applies

$$P(\tau) = e^{-\tau} \quad (4.6)$$

By correctly sampling many random $\tau$, it is possible to get $e^{-\tau}$ after many samplings

$$\xi = \int_{0}^{\tau} e^{-\tau} d\tau \quad (4.7)$$

Solving Equation 4.7 above yields

$$\tau = -\ln \xi \quad (4.8)$$

where $\tau$ is the optical depth and $\xi$ is a random number between [0,1]

By sampling random $\tau$ as described above, a photon pathlength, $S$ – the distance to an interaction location – may then be computed as follows

$$\tau = \int_{0}^{S} \mu_t ds \quad (4.9)$$

where $\mu_t$ is the total attenuation coefficient and $S$ is the physical distance to an interaction site.
After a photon has travelled a randomly chosen $\tau$, it is then either scattered or absorbed. The scattering probability is defined by the albedo, $a$, which can be expressed as

$$a = \frac{\mu_s}{\mu_s + \mu_a} \quad (4.10)$$

where $\mu_s$ is the scattering coefficient and $\mu_a$ is the absorption coefficient.

If a photon is absorbed, photon energy is deposited in the medium and can be re-emitted as fluorescence. The total attenuation coefficient, $\mu_t = \mu_s + \mu_a$. Typical values for $\mu_a$, $\mu_s$ and $\mu_t$ of the skin at 630 nm are 1.5 cm$^{-1}$, 150 cm$^{-1}$ and 151.5 cm$^{-1}$, respectively. For these parameters, an optical depth, $\tau = 1$, occurs at a depth of approximately 0.007 cm in the skin. A photon’s position on our code is given by three spatial Cartesian coordinates $(x, y, z)$ which are used to trace photon movement, and a photon’s direction of travel is described by three directional cosines

$$n_x = \sin \theta \cos \phi$$
$$n_y = \sin \theta \sin \phi$$
$$n_z = \cos \theta \quad (4.11)$$

The scattering of a photon is described by two directional angles; the deflection angle, $\theta$ and the azimuthal angle, $\phi$, as shown in Figure 4.1 [14]. The Henyey-Greenstein scattering phase function [15] is adopted to approximate scattering in tissue and is mathematically expressed in the form

$$P(\theta) = \frac{1}{4\pi} \frac{1 - g^2}{[1 + g^2 - 2g \cos(\theta)]^{3/2}} \quad (4.12)$$

where $P(\theta)$ is the probability distribution function and $\theta$ is the deflection angle in radians, which the photon is scattered through with respect to the incident direction.
g is defined as

\[ g \equiv \langle \cos(\theta) \rangle = \int_0^\pi P(\theta) \cos(\theta) 2\pi \sin \theta d\theta, \]  

(4.13)

where

\[ \int_0^\pi P(\theta) 2\pi \sin \theta d\theta = 1 \]  

(4.14)

g is known as the scattering anisotropy factor and affects the angular distribution and therefore the amount of forward direction maintained by the photon. g is represented by values between -1 and 1, which indicate backward-directed and forward directed scattering, respectively, while 0 corresponds to isotropic scattering. Jacques et al. [16] have shown that scattering within skin tissue can be represented quite well by the Henyey-Greenstein scattering phase function. Typical values for g are in the region of 0.7 to 0.95. Given that scattering in tissue is predominately forward directed, the following equation is used to generate scattering angles.

\[ \cos \theta = \frac{1}{2g} \left\{ 1 + g^2 - \left[ \frac{1 - g^2}{1 - g + 2g\xi} \right]^2 \right\} \quad \text{if} \quad g \neq 0 \]  

(4.15)

For isotropic scattering, sin \( \theta \) is sampled over the range 0 to \( \pi \), while \( \phi \) is sampled over the interval 0 to \( 2\pi \).

\[ \cos \theta = 2\xi - 1 \quad \text{if} \quad g = 0 \]  

(4.16)

and

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

(4.17)
Figure 4.1 The deflection angle, $\theta$, influences the amount of forward direction $\cos(\theta)$, maintained by a photon after a scattering event (Adapted from Ref. 14).

Snell’s law represents the relationship between the angle of incidence $\theta_i$ and the angle of transmission $\theta_t$ for a photon incident on an interface between two media $(n_1, n_2)$ with different refractive indices and is expressed as follows

$$n_i \sin \theta_i = n_t \sin \theta_t$$  \hspace{1cm} (4.18)
Fresnel’s equations may be used to describe the reflection and refraction that occurs when a photon propagates from one medium to another medium of varying refractive index [17]. The probability that a photon is internally reflected at the surface is given by the Fresnel reflection coefficient, \( R(\theta_i, \theta_t) \) [18] as expressed below

\[
R(\theta_i, \theta_t) = \frac{1}{2} \left[ \frac{\sin^2(\theta_i - \theta_t) + \tan^2(\theta_i - \theta_t)}{\sin^2(\theta_i + \theta_t) + \tan^2(\theta_i + \theta_t)} \right]
\] (4.19)

Again, a random number is used to determine whether the photon is internally reflected or transmitted. If \( \zeta < R(\theta_i, \theta_t) \) the photon is internally reflected, otherwise the photon is transmitted. This enables the model to take into account photons that either escape the tissue or if internally reflected back proceed on their random walk with an updated position and direction. When a photon is absorbed it deposits energy in the tissue. Photon absorption is computed by the following equation

\[
Q = \frac{L}{N \Delta V} \sum_i \mu_a S_i
\] (4.20)

where \( Q \) is the energy absorbed per cubic centimeter per second, \( L \) is the luminosity – energy per second – in Watts, \( \Delta V \) is the volume of the cell, \( \mu_a \) is the absorption coefficient and \( S_i \) is the distance along the photon path in a cell.

The fluence rate, \( \psi \), in a specific cell in the grid system is given by

\[
\psi = \frac{L}{N \Delta V} \sum_i S_i
\] (4.21)

where \( L \) is the luminosity – energy per second – in Watts, \( N \) is the total number of photons launched in the simulation, \( \Delta V \) is the volume of the cell and \( \sum_i S_i \) is the sum of the photon pathlengths in a cell.
As described previously in Chapter 1, Section 1.3.2, fluorescence photobleaching has an important role in PDT treatment planning and should be considered when developing dosimetry in PDT. Pertinent equations involving photobleaching were incorporated into the MCRT model. Photobleaching as described previously by Equation 1.2 was simulated by the MCRT model. If excitation photons are absorbed by PpIX, the absorbed photons are re-emitted as fluorescent photons, which are weighted by the fluorescence yield, i.e. 5%. A threshold value of approximately $8.60 \times 10^{17}$ $\text{O}_2$ molecules generated per cm$^3$ by a porphyrin photosensitiser in tumours was used as the threshold photodynamic dose, $\text{PD}_T$ [19]. Following the work of Farrell et al. [20], we assumed that the local yield of $\text{O}_2$ generation following absorption of photons by the photosensitiser defined the PDD and tissue necrosis was assumed to occur when $\text{PDD} > \text{PD}_T$.

The PDD as defined earlier in Chapter 1, Section 1.3.2, is an important dosimetric parameter. A threshold photodynamic dose, $\text{PD}_T$, leads to tissue necrosis if the number of $\text{O}_2$ molecules generated by the photosensitiser per unit volume of tumour tissue exceeds a certain value. The PDD is directly related to the photosensitiser concentration and the light fluence rate, as is shown by the following equation

$$\frac{d\text{PDD}}{dt} = \gamma_0 \Psi(x, y, z)C(x, y, z, t)$$

(4.22)

where $\text{PDD}$ is expressed in units ($\text{O}_2 \text{cm}^{-3}$), $\gamma_0$ ($\text{O}_2 \text{J}^{-1}$) is the constant quantum yield for singlet oxygen generation when the photodynamic therapy process was not limited by the availability of oxygen concentration, $\Psi(x, y, z)$ is expressed in units (mWcm$^{-2}$) and $C(x, y, z, t)$ in units cm$^{-1}$ [20].

Although computational time can be an issue owing to the statistical nature of MC methods, it is possible to achieve accurate profiles of light distributions in tissue [21]. The above mentioned equations were instrumental for obtaining the results presented both in this chapter and throughout this thesis.
4.2.1 Description of the Monte Carlo Radiation Transfer Model

The MCRT model used throughout this research was based on a three dimensional (3D) cube shaped geometry [22-24] and removed the assumption of an optically semi-infinite tissue volume. This MCRT code was written and developed to simulate scattering, absorption, fluorescence and photobleaching processes present in PDT. Also, an algorithm was incorporated into the code to determine the production of singlet oxygen. The MCRT model was implemented in Fortran 77 and simulations were performed on a computer with a processor speed of 1.5 GHz, with each simulation taking approximately 30 minutes to complete 1 million input photons. Simulations were performed on a 3D Cartesian grid divided up into 101 x 101 x 101 grid cells. Each grid cell was represented by a 3D array location \((i, j, k)\) and could be assigned varying wavelength-dependent optical properties. The optical properties are characterised by \(\mu_s, \mu_a, g\) and \(n\), as described above.

A flowchart representing the movement of a photon is illustrated in Figure 4.2. Each excitation photon packet is launched from the source and propagates through the cube grid, whilst undergoing scattering or absorption. The photon is directed downwards into the grid – with defined optical properties – and travels until it reaches its first interaction location, as given by Equation 4.8. Consequently, it is either scattered or absorbed, the probability of which is determined by the albedo \(a\), where a larger value of \(a\), corresponds to a more highly scattering environment. If the photon is scattered in the grid, it will therefore scatter into a new direction characterised by the Henyey-Greenstein scattering phase function. Scattering will continue until the photon is eventually absorbed or exits the grid. The photon is binned according to its angle when exiting the grid and position, which produce a 2D image, and wavelength, which generates a spectrum.

Alternatively, photons may be internally reflected at the surface boundary undergoing further scattering or absorption. If absorption occurs, the photon contributes to the amount of energy deposited in the corresponding cell. In addition to simulating the generation and propagation of an excitation photon, a fluorescence photon can also be generated if an excitation photon is absorbed by PpIX in the grid, which has defined wavelength-dependent optical properties. Fluorescence photons are isotropically emitted and traverse the grid at the fluorescence emission wavelength. Furthermore, the fluorescence photons are tagged by assigning a number to them in
the code, which enables their depth of origin to be recorded. Ultimately, when the tagged fluorescence photons exit the grid at the surface, they are binned according to their angle when exiting the grid and position, and also the depth where they originated from within the grid. Also, the exiting fluorescence may be recorded as a spectrum. Once an excitation photon is absorbed or exits the grid, a new photon is introduced from the source. This is repeated and the simulation ends when the total specified number of photons has been launched.

Figure 4.2 Movement of a single photon by the MCRT method.
4.3 Experimental Validation of the MCRT Model

The propagation of light in a scattering turbid media as described by the MCRT model was investigated enabling the accuracy of the model to be evaluated. The objective was to compare MCRT model outputs with results from a set of light scattering laboratory experiments. Scattering laser beam profiles obtained from the experimental measurements were compared against simulated scattering laser beam profiles generated by the MCRT model.

4.3.1 Intralipid 20 %

The direction of light scatter is predominately forward directed when it propagates through a solution of Intralipid 20 % as the particle size is close to the size of wavelengths in the visible region of the EM spectrum [2]. Therefore, Intralipid 20% was used experimentally to mimic the light scattering characteristics of biological turbid media, such as the skin. To avoid confusion, Intralipid 20 % will be simply stated as Intralipid. This stock solution is an aqueous suspension of purified soyabean oil, egg phospholipids, glycerol anhydrous and water. In line with previous work undertaken by Driver et al., [2] varying concentrations of Intralipid ranging from 0.1 % to 0.8 % were aliquoted. This was achieved by diluting the stock solution with distilled water. Firstly, 1 ml of the stock solution was added to 99 ml of distilled water, yielding 100 ml of a 1 % (volume/volume) Intralipid solution. Secondly, 1 % Intralipid solutions were diluted by adding various concentrations of distilled water. For example, to obtain a 0.2 % Intralipid solution, 1 ml – from the 100 ml – of the 1 % Intralipid solution was loaded – with a pipette – into a beaker followed by 4 ml of distilled water, producing 5 ml of a 0.2 % Intralipid solution. Flock et al., [25] reported the anisotropy factor, g, of Intralipid to be 0.7.

4.3.2 Experimental Design

An experimental study of light propagation in the highly scattering agent – Intralipid – is presented next. The experimental set-up is shown below by both a photograph (Figure 4.3) and a simplified schematic diagram (Figure 4.4). Firstly, a control experiment was carried out using a special optical glass cuvette loaded with distilled water. Light was directed perpendicularly onto the front face of the cuvette and light exiting the back face was recorded by a detector connected to a power-meter. The
detector was positioned as close as possible to the cuvette. Initially, a razor blade blocked the laser beam so that no light fell incident onto the detector. Room lights were switched off to minimise artefacts from ambient lighting. The razor blade was then transversely scanned – in incremental steps of 50 µm – across the back face of the 1 cm cuvette using a translational stage with a micrometer screw gauge. The detector measured the relative power ($\mu W$) as a function of razor blade position. This enabled a knife-edge scan to be plotted as shown in Figure 4.5 (a). By calculating the derivative of the knife-edge scan as shown in Figure 4.5 (b) it was possible to reconstruct the original laser beam profile. The beam diameter – defined by the FWHM – was 0.035 cm. Subsequently, the cuvette was loaded with increasing Intralipid concentrations and the procedure was repeated. Firstly, a Helium-Neon (HeNe) laser – emission wavelength (632 nm), power (0.700 mW) and beam diameter (0.035 cm) – was used. Next, a Millennia Xs laser – emission wavelength (532 nm), power (16.4 mW) and beam diameter (0.122 cm), followed by a Toptica ibeam semiconductor laser diode – emission wavelength (405 nm), power (33.5 mW) and beam diameter (0.22 cm) were used as experimental light sources. Each laser had a Gaussian beam profile. Laser beam profiles – indicative of how the incident beam changed as it traversed the Intralipid solutions – were determined for a range of Intralipid solutions and wavelengths. Each Intralipid solution was made up in distilled water.
Figure 4.3 Laboratory set-up of the knife-edge technique for the study of light propagation in Intralipid.

Figure 4.4 Simplified schematic set-up of the knife-edge technique for the measurement of scattered knife-edge profiles [26].
Figure 4.5 (a) Knife-edge scan recorded from the control experiment, where the cuvette was loaded with distilled water; (b) derivative of the knife-edge scan, which enabled the original laser beam profile to be constructed in one dimension.
4.3.3 Simulation Design

It was necessary to use the correct model inputs for the simulations in order to ensure an accurate representation of the experimental measurements obtained with the Intralipid solutions. The MCRT code was set up with grid dimensions of 10 mm x 10 mm x 10 mm \((x, y, z)\) as a 10 mm x 10 mm \((x, z)\) cuvette was used in the experimental set-up. As shown in Figure 4.6, photons were injected into the bottom of the grid with a Gaussian beam profile analogous to the experimental set-up as shown in Figure 4.3 and the position was sampled in the model by the following equation

\[
FWMH = 2\sqrt{\ln 2}\sigma
\]  

where \(\sigma\) is the standard deviation

\(g\) was set as 0.7 [25], \(a\) was taken as 0.999 and \(\mu_t\) was varied depending on the experimental Intralipid solution used. The beam diameter and \(\mu_t\) in the model were altered depending on the experimental laser used. One million photons were launched for each simulation as this gave adequate signal to noise in the simulations. The transmitted light photons detected at the top surface of the grid were saved in a matrix. By summing the photons in the image – similar to the experimental knife-edge technique – it was possible to obtain a simulated knife-edge scan and consequently the derivative of the scan. Firstly, the opacity of the grid was set to zero (\(\mu_t = 0\)) resulting in an optical depth, \(\tau\), of zero. This represented no scattering or absorption in the grid and all the photons propagated through the grid until exiting at the top. Figure 4.7 (a) compares this simulated knife-edge profile to the measured knife-edge profile recorded from the control experiment. Depending on the optical depth of the grid, photons will undergo varying scattering and absorption events, thus propagating the grid differently, giving rise to different scattered knife-edge profiles. The absorption and scattering coefficients are related to the optical depth by \(\tau(\lambda) = \mu_t(\lambda)S\). The simulations were repeated for the other wavelengths and beam diameters, and appropriately matching total attenuation coefficients were used in the model to compare to each % Intralipid concentration.
4.3.4 Experimental Validation Results

A series of scattering laser beam profiles were constructed relating to 0 %, 0.2 %, 0.4 % and 0.8 % Intralipid concentrations. By executing a series of simulations where the total attenuation coefficient parameter, $\mu_t$, was altered, it was possible to match a specific simulation with an experimental profile. It was apparent that increasing the % Intralipid concentration in the experiments led to more scattering in the wings of the laser beam. In order to reproduce this observation by the MCRT model, it was necessary to increase $\mu_t$, in the simulations, which in turn led to an increase in scattering in the wings of the beam. For a homogeneous medium, the optical depth is equal to the total attenuation coefficient multiplied by the photon pathlength. Different scattered knife-edge profiles are presented in Figure 4.7 – the red lines...
indicate the experimental data, while the black are indicative of the simulation outputs – and illustrate that more scattering was evident when the total attenuation coefficient – which is analogous to the % Intralipid concentration – increased. Moreover, the relative power (arb. units) decreased as the total attenuation coefficient, $\mu_t$, increased. Similar effects were observed when the abovementioned procedure was repeated with different wavelengths, namely, 532 nm (Figure 4.8) and 405 nm (Figure 4.9). Overall, this comparison between the experimental data and the corresponding simulated data shows that the scattered laser beam profiles are in good agreement with each other. Figure 4.10 (a) compares normalised simulated knife-edge profiles recorded using 632 nm and illustrates more clearly the fact that scattering increased as the total attenuation coefficient increased. This was repeated for 532 nm (Figure 4.10 (b)) and 405 nm (Figure 4.10 (c)). As the wavelength decreased, the total attenuation coefficient increased and more scattering was apparent, highlighting the fact that the total attenuation coefficient is wavelength dependent. Light of varying wavelengths are scattered differently by the particles in the Intralipid solution and this can be described by Mie scattering.

The incident beams were modified depending on the concentration of scatterers and these observations are of particular interest in PDT. Moreover, the higher total attenuation coefficients associated with the 405 nm laser resulted in a noticeably lower transmission of light when compared with the other 632 nm and 532 nm lasers. The penetration of light – with different wavelengths – through Intralipid was examined with a spectrophotometer by inserting varying concentrations of Intralipid and recording the corresponding % Transmission. Figure 4.11 illustrates % Transmission plotted as a function of % Intralipid Concentration. Overall the % Transmission decreased as a function of increasing % Intralipid concentration and decreasing wavelength.
Figure 4.7 Scattered knife-edge profiles – red lines indicate the experimental data, while the black are indicative of the simulation outputs – produced by the 632 nm laser; (a) 0 % Intralipid concentration compared to $\mu_t = 0$; (b) 0.2 % Intralipid concentration compared to $\mu_t = 2.25$; (c) 0.4 % Intralipid concentration compared to $\mu_t = 4.65$ and (d) 0.8 % Intralipid concentrations compared to $\mu_t = 8.5$. Moreover, the relative power (arb. units) decreased as the % Intralipid concentration and the total attenuation coefficient, $\mu_t$, increased.
Chapter 4 – Monte Carlo Radiation Transfer Modelling

Figure 4.8 Scattered knife-edge profiles – red lines indicate the experimental data, while the black are indicative of the simulation outputs – produced by the 532 nm laser; (a) 0 % Intralipid concentration compared to $\mu_t = 0$; (b) 0.2 % Intralipid concentration compared to $\mu_t = 3$; (c) 0.4 % Intralipid concentration compared to $\mu_t = 5.25$ and (d) 0.8 % Intralipid concentrations compared to $\mu_t = 9$. Moreover, the relative power (arb. units), decreased as the % Intralipid concentration and the total attenuation coefficient, $\mu_t$, increased.
Figure 4.9 Scattered knife-edge profiles – red lines indicate the experimental data, while the black are indicative of the simulation outputs – produced by the 405 nm laser; (a) 0 % Intralipid concentration compared to $\mu_t = 0$; (b) 0.2 % Intralipid concentration compared to $\mu_t = 5.5$; (c) 0.4 % Intralipid concentration compared to $\mu_t = 8$ and (d) 0.8 % Intralipid concentrations compared to $\mu_t = 11$. Moreover, the relative power (arb. units) decreased as the % Intralipid concentration and the total attenuation coefficient, $\mu_t$, increased.
Figure 4.10 Normalised simulated knife-edge profiles with increasing $\mu_s$, recorded using (a) 632 nm; (b) 532 nm and (c) 405 nm and illustrates more clearly the fact that scattering increased as the $\mu_s$ increased. As the wavelength decreased, the total attenuation coefficient increased and more scattering was apparent, highlighting the fact that the total attenuation coefficient is wavelength dependent.
Figure 4.11 % Transmission plotted as a function of % Intralipid Concentration. Overall the % Transmission decreased as a function of increasing % Intralipid concentration and decreasing wavelength.

4.4 Theoretical Validation of the MCRT Model

Next, the performance of the MCRT model was compared against results published by Keijzer et al. [3] and Jacques et al. [4]. Firstly, fluence rate contours pertaining to artery tissue previously published by Keijzer et al. [3] were used in a theoretical validation of our MCRT model. A second theoretical validation was carried out whereby the PDD simulated by Jacques et al. [4] was compared to the PDD computed by the model. The results were assessed and the validity of the model examined.
4.4.1 Fluence Rate Contours in Artery Tissue

While it is practical to calculate the irradiance delivered at a tissue surface, it is difficult to determine the fluence rate and therefore the distribution of light within tissue. An accurate description of theoretical light distributions in tissue through fluence rate contours may be computed by the MCRT method. Fluence rate profiles are indicative of how light may be distributed inside tissue as a function of position. An estimation of the fluence rate in tissue can yield information pertaining to the depth of light penetration and the light dose within the tissue and is consequently useful in light dosimetry planning [27].

Keijzer et al. [3] individually simulated collimated incident beams with diameters of 0.2 mm, 1 mm and 4 mm each with a power density of 1 W/cm². Fluence rate contours were recorded enabling light distributions of 476 nm light in human artery tissue to be represented. Employing their optical properties – $\mu_a = 6 \text{ cm}^{-1}$, $\mu_s = 414 \text{ cm}^{-1}$, $\mu_t = 420 \text{ cm}^{-1}$, $g = 0.91$ and $n = 1.37$ –, grid set-up and mis-matched boundary conditions, simulations were performed using the MCRT model. For the purposes of this study, the MCRT model was set up with grid dimensions of 6 mm x 6 mm x 6 mm $(x, y, z)$. One million photons were launched for each simulation. The beams were centrally located and normally incident onto a tissue surface of 6 mm with a depth of 1.5 mm. Fluence rate distribution patterns simulated by the MCRT model as a function of position in artery tissue were compared with those presented by Keijzer et al. [3]. Figure 4.12 (a) – (c) illustrates our MCRT simulated fluence rate contours – which are slices through the grid – from a finite width 0.2 mm, 1 mm and 4 mm diameter laser beam, respectively. The contours depicted by Keijzer et al., [3] were overlayed onto Figure 4.12 (a) – (c) for a quantitative comparison. Ultimately, Figure 4.12 (a) – (c) illustrates good agreement between the results generated by our model and those published by Keijzer et al. [3]. Comparison of the data sets show that the MCRT model is able to predict the characteristic distribution of light in artery tissue and the change thereof that accompanies varying beam diameters. In light of these results, the MCRT model could be further developed for PDT applications.
Figure 4.12 Light distributions in artery tissue for collimated incident beams with diameters of (a) 0.2 mm; (b) 1 mm and; (c) 4 mm. The black and white contours of Keijzer et al. [3] overlayed onto the coloured MCRT simulated contours.
4.4.2 Photodynamic Dose Contours in Subcutaneous Rat Tumours

As described previously in Section 4.2, the MCRT model was modified to simulate photobleaching. This must be considered for investigations of clinical PDT treatments. For the purposes of theoretical validation here, outputs from the further developed MCRT model were compared to those simulated by Jacques et al. [4]. Using a PDT dose model, Jacques et al. [4] reported on the significance of the role of photobleaching in PDT treatment planning and provided an insight to the threshold photodynamic dose ($\text{PD}_T$) – which leads to tissue necrosis – at specific treatment times and depths in a tumour. This implicit dosimetry approach in PDT – using fluorescence photobleaching kinetics of a photosensitiser as a dose metric – was adopted for the MCRT model.

As before in Section 4.4.1, the specified tissue optical parameters were adopted in the MCRT model. At 630 nm – $\mu_a = 0.23 \text{ cm}^{-1}$, $\mu_s' = 21 \text{ cm}^{-1}$ and $n = 1.38$. Figure 4.13 illustrates the PDD generated after MCRT simulations were performed in time steps of 40 seconds until approximately a PDT simulated treatment time of ~ 1 hour (time = 3620 seconds) was represented. One million photons were launched for each simulation. The results were compared to the reported PDD as a function of depth and time in the presence of photobleaching by Jacques et al. [4] and were found to be in close agreement. A maximum PDD value of $1.753 \times 10^{18}$ was obtained by Jacques et al. [4] while the MCRT model accomplished $1.769 \times 10^{18}$. These results confirm that the MCRT model is able to determine the PDD administered to tissue during PDT treatment as previously described in the literature. The MCRT model is now in a position to address specific questions concerning clinical PDT treatments.
Figure 4.13 The PDD as a function of depth and time in the presence of photobleaching during a simulated PDT treatment performed by the MCRT model. In line with Jacques et al. [4], a threshold value of approximately $8.60 \times 10^{17} \text{O}_2/\text{cm}^3$ ( = $PD_T$) generated by a porphyrin photosensitiser in tumours was used and indicated by the horizontal line.

4.5. Conclusion
The MCRT technique can trace many individual photon trajectories offering a physical, accurate description of light propagation in tissue [28] without restrictions in optical properties and has the major advantage of handling complex and realistic tissue geometries [11]. It is statistical in nature and requires a large number of launched photons in order to build up an accurate representation of the net distribution of photon paths. MC simulations can generate photon paths pertaining to the
propagation of light in tissue, which effectively provides information relating to where the light has travelled in the tissue [3]. While this technique is computationally intensive, it yields information close to reality. As a result, MCRT modelling is often used for comparing the accuracy of other models, which are also concerned with the transport of light in tissue [29]. Knowledge of how light is distributed through highly scattering material is important in light-based cancer treatments, such as PDT. The development and validation of this mathematical model by both an experimental and theoretical approach outlined a rigorous assessment of test parameters, which in turn, evaluated the accuracy and suitability of the model for PDT applications.

Knife-edge profiles were determined when laser light was directed perpendicularly onto a cuvette loaded with specific concentrations of Intralipid. Due to the dynamic range of the power-meter, some of the experimental data is noisy. However, the results offer a clear and significant outcome; it was possible to obtain different scattered knife-edge profiles, whereby each incident beam was modified depending on the concentration of scatterers present in the Intralipid solutions. Overall, the comparison between the experimental data and the corresponding simulated data showed that the theoretical and laboratory scattered knife-edge profiles were in good agreement with each other.

From the theoretical validation standpoint, the MCRT model results were found to be in good agreement with those published in the literature, when incorporating the specified optical parameters [3, 4]. This ensured that the MCRT model was capable of offering accurate representations of, for example, light fluence rate, scattering and absorption, reflection, transmission and the PDD, which can be used to extract pertinent information about PDT treatment occurring within the tissue. The validation results presented above approves the MCRT model for dosimetric investigations of clinical PDT treatments.
4.6 References


Chapter 5 – Monte Carlo Radiation Transfer Modelling in Topical PDT

5.1 Introduction
MCRT modelling – as outlined and described in the preceding chapter – was applied to PDT in order to answer specific questions pertaining to treatment variables. This chapter will present PpIX fluorescence measurements acquired from patients presenting with sBCC during PDT treatment, facilitating \textit{in vivo} photobleaching to be monitored. One of the main purposes for this study was to simulate the transmission of light through tissue so as to assess the light dose during PDT and determine the fluorescence emission on the skin surface, which originated from varying depths within the tumour. MC simulations taking into account photobleaching, were performed on a 3D cube grid, which represented the treatment geometry. Consequently, it was possible to determine the spatial and temporal changes to the origin of collected fluorescence and generated singlet oxygen.

PDT is recognised as an effective treatment of NMSCs [1], however further optimization for PDT treatment is required. The work of Wilson et al. [2], has shown that by employing a technique such as implicit dosimetry, fluorescence photobleaching may be used as a dose metric. Fluorescence and photobleaching measurements can contribute significantly to the development of photodynamic therapy dosimetry [3,4]. Robinson et al. [5], have suggested that a reduction in photosensitiser fluorescence caused by photobleaching is indicative of the photodynamic dose, PDD, administered. It is therefore possible to monitor, via photosensitiser fluorescence measurements the amount of drug in the tissue that has photobleached during PDT and relate it to the PDD [6]. Optical fluorescence spectroscopy offers rapid diagnostic information by using light-tissue interactions [7]. It is used for the early detection of cancerous and pre-cancerous lesions and monitoring of PDT treatments. The interactions of light with biological tissue causes light absorption and scattering as the photons propagate through the tissue. In order to fully optimize clinical PDT treatments, new approaches must be taken to model the process, which as well as taking into account the photochemical behavior discussed above, also take into account the propagation of both the treatment wavelengths and

the subsequent fluorescence wavelengths used for monitoring within tissue. One approach is to use radiation transfer simulations.

In this study *in vivo* ALA-induced PpIX fluorescence measurements have been recorded during clinical PDT in humans from the surface of sBCC. Using this diagnostic, a photobleaching dose constant, $\beta$, of ALA-induced PpIX fluorescence was obtained for patients presenting with sBCC. These results were then incorporated into a 3D MCRT model that enabled predictions to be made about the efficacy of treatment in PDT. In particular, we have used our model to address the important question of how long after surface PpIX fluorescence has diminished the PDT treatment is still effective and to what depths below the surface is effective treatment provided. To more accurately represent clinical PDT, in this chapter we consider a model with a tumour of finite size embedded and surrounded in normal tissue and subjected to a finite uniform superficial irradiation. To the best of our knowledge we believe that this is the first time that data obtained directly from clinical PDT treatments has been combined with a 3D MCRT model to enable modeling of a fully 3D tumour phantom of finite size embedded in normal tissue.

### 5.2 Materials and Methods

#### 5.2.1 Clinical Topical-Photodynamic Therapy

As previously described in Chapter 3, Section 3.2, PDT treatments were carried out at the Photobiology Unit, Ninewells Hospital and Medical School, Dundee, using the photosensitiser pro-drug, ALA. Formal consent was obtained from the patients before the study was undertaken. ALA was topically applied to the lesional area. Following a 6 hour incubation period, one skin lesion per patient was treated. Data from six patients was used in this study.

#### 5.2.2 Clinical Fluorescence Measurements from Superficial Basal Cell Carcinomas

As mentioned earlier, the uptake and conversion of ALA to PpIX in diseased tissue leads to increased levels of PpIX in lesions. A high fluorescence ratio of tumour to surrounding tissue allows for demarcation of the tumour. In this study, fluorescence spectra were acquired using the fibre-coupled fluorescence spectroscopy system – OBS – which has been previously described in Chapter 2, Section 2.3. PpIX
fluorescence was induced in the skin and spectra were recorded by placing the probe perpendicularly in contact with the tissue. From each individual lesion, eight fluorescence spectra were recorded before ALA application, immediately before treatment, approximately half-way during treatment and immediately after treatment. The mean spectrum was then calculated from each set of eight spectra for each lesion. Treatment was stopped briefly for ~1 minute to allow for the halfway measurements to be carried out. All measurements were performed in a darkened room to minimise artifacts from ambient room light.

5.2.3 Description and Validation of the Monte Carlo Radiation Transfer Model

It was possible to account for the generation and propagation of excitation photons, and of fluorescence photons emanating from a tumour embedded in normal skin tissue. The code also incorporated an algorithm in the code to determine the production of singlet oxygen in the tumour. Whilst other MCRT simulations of photon transport and fluorescence have been performed in multilayered tissues [8,9] this 3D MCRT code can compute light distributions for a clinical PDT geometry where a tumour is embedded in normal skin tissue. Earlier studies have used this type of geometry to model the distribution of light within a tumour [10,11]. Our code however also incorporates different optical properties for normal and tumour tissue and the addition of a photosensitiser, namely, PpIX into the tumour. Furthermore, photobleaching has also been included. This enables the efficacy of PDT to be investigated. Table 1 shows a list of important symbols used throughout this work. In particular, within our code, $\mu_a$ and $\mu_s$ are the tissue absorption and scattering coefficients, respectively, which can be varied depending on the depth within our phantom, and $g$ is the scattering anisotropy factor. Our model is based on a 3D cube shaped geometry [12-14].

To achieve statistical significance, simulations were performed with $10^8$ photons, on a 3D Cartesian grid geometry, as illustrated in Figure 5.1. The tumour was placed at the surface of the skin. The total dimensions of the cube were taken to be 20 mm x 20 mm x 20 mm (x,y,z) and the modeled tumour had a radius of 5 mm and penetrated 4 mm into the cube. In our simplified situation, the tumour was represented by a cylinder and placed at the centre of the normal skin tissue, which was represented by a cube. Our 3D representation provides a reasonably accurate analogue
to the situation present in clinical PDT, where both the tumour and some of the surrounding normal skin tissue are superficially irradiated by a finite beam. As shown in Chapter 4, Section 4.4, the MCRT code was validated by comparing a range of simulations to the results generated by Keijzer et al. [15] where light distributions in artery tissue were examined and Jacques et al. [16] when photobleaching was taken into account.

Figure 5.1 *Three – dimensional (3D) MCRT model geometry, where x, y are mutually orthogonal axes in the skin surface and z represents depth within the skin tissue.*
Table 5.1 Listing of symbols used throughout this work.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu_a(x, y, z))</td>
<td>absorption coefficient (cm(^{-1}))</td>
</tr>
<tr>
<td>(\mu_s(x, y, z))</td>
<td>scattering coefficient (cm(^{-1}))</td>
</tr>
<tr>
<td>(g)</td>
<td>scattering anisotropy factor</td>
</tr>
<tr>
<td>(a)</td>
<td>(\frac{\mu_s}{\mu_a + \mu_s})</td>
</tr>
<tr>
<td>(I)</td>
<td>surface irradiance (mW/cm(^2))</td>
</tr>
<tr>
<td>(\Psi(x, y, z))</td>
<td>fluence rate (mW/cm(^2))</td>
</tr>
<tr>
<td>(C(x, y, z, t))</td>
<td>PpIX concentration (cm(^{-1}))</td>
</tr>
<tr>
<td>(\beta)</td>
<td>photobleaching dose constant (J/cm(^2))</td>
</tr>
<tr>
<td>(\tau_{PB})</td>
<td>photobleaching time constant (seconds)</td>
</tr>
<tr>
<td>(z)</td>
<td>depth in tissue (cm)</td>
</tr>
<tr>
<td>PDD</td>
<td>photodynamic dose ((1^\circ)O(_2)/cm(^3))</td>
</tr>
</tbody>
</table>

5.2.4 Monte Carlo Radiation Transfer Model Assumptions

With clinical PDT in mind it was necessary to make some assumptions in our model. We have assumed mis-matched boundary conditions and in a similar method to Farrell et al. [6], that the tumour had a homogeneous distribution of PpIX. Photobleaching has been previously described in Chapter 4, Section 4.2.

5.2.5 Application of the MCRT Model to Clinical Photodynamic Therapy

On the basis on our assumptions, we modeled a clinical situation, i.e., the treatment itself and the fluorescence measurements undertaken during treatment requiring two different sets of simulations to be undertaken. The former consisted of simulations where the singlet oxygen produced in the tumour was modeled as a function of depth and time, while the latter included modeling the PpIX fluorescence detected at particular time points in the treatment. In this way our model could provide information about both the fluorescence information used as a diagnostic and the possibility of tumour cell kill. We sampled the fluorescence emission in the code from
clinically measured ALA-induced PpIX fluorescence spectral data. This approach is different from other models where only excitation and fluorescence emission wavelengths have been used rather than full spectral data [16,17]. Figure 5.2 depicts the optical properties used in the MCRT simulations, which were taken from data published in the literature [18].

Figure 5.2 Spectral characteristics and model inputs of (a) normal skin and basal cell carcinoma scattering coefficients; (b) normal skin and basal cell carcinoma absorption coefficients. Data from Salomatina et al. [18].

The absorption coefficient of PpIX as a function of wavelength was shown previously in Chapter 1, Section 1.2, Figure 1.3. This illustrates our absorption coefficient for PpIX as a function of wavelength. A stock solution of PpIX in Dimethylsulfoxide
(DMSO) was produced with a concentration of 45 μg/ml. The solution was then placed in a cuvette where a spectrophotometer (Hitachi Spectrophotometer U-3010) was used to measure the optical absorbance and the absorption coefficient for PpIX was determined to be 0.06 cm\(^{-1}\) at 630 nm, using an extinction coefficient for PpIX, \(\varepsilon_{630\,\text{nm}} = 0.0014 \, \mu\text{g/ml}^{-1}\text{cm}^{-1}\). This is within the range of values published in the literature [6].

For the PDT treatment simulations, we based the inputs for our model on the well-established treatment parameters used in the Scottish Photodynamic Therapy Centre, Ninewells Hospital & Medical School, Dundee. The entire top surface of the cube, was irradiated uniformly, at the treatment wavelength of 632 nm. A surface irradiance, \(I\), of 82 mW/cm\(^2\) was delivered over a simulated treatment time of approximately 30 minutes, thus administrating a simulated total treatment light dose (LD) of 150 J/cm\(^2\). The photons propagated through the normal skin and tumour using the scattering and absorption coefficient values at this wavelength. After photon absorption in the tissue, the amount of energy deposited was computed. The energy absorbed in the tumour was determined at each time step, corresponding to increments of 40 seconds. Based on our assumptions of the PDD, it was possible to infer the 3D distribution of photons and hence 3D distribution of singlet oxygen production in the tumour as a function of LD.

For the PDT fluorescence simulations, PpIX fluorescence photons were induced at specific stages during the PDT treatment simulations. The entire top surface of the cube and cylinder was irradiated uniformly, at the fluorescence excitation wavelength of 400 nm. Fluorescence photons that exited the top surface were tracked from their place of origin and it was then possible to know exactly where they came from inside the tumour. After each PDT treatment simulation was carried out, the PDT fluorescence simulation followed immediately afterwards. In other words, the two simulations were run simultaneously for given time steps, i.e., every 40 seconds, up to ~ 1800 seconds. In this manner, PpIX fluorescence was detected in the tumour as a function of depth and LD in the presence of photobleaching.
5.3 Results

5.3.1 In vivo ALA-induced PpIX Fluorescence Spectra

Figure 5.3 illustrates the mean fluorescence of the eight spectra from each of the six patients before the application of the ALA cream. As depicted by Figure 5.3 (a) collected peak autofluorescence signals ~ 500 nm are highly variable between patients. Collected PpIX fluorescence signals ~ 635 nm can also vary between patients as illustrated in Figure 5.3 (b).

![Figure 5.3 Baseline PpIX fluorescence intensity – $F_{PpIX}$ – spectra recorded before ALA application for 6 patients and the mean is depicted in bold, (a) spectral region showing tissue autofluorescence; (b) spectral region where PpIX fluorescence peak will feature.](image)

Figure 5.4 presents PpIX fluorescence spectra non-invasively measured in six different patients as a function of time during treatment. Note that in both sets of figures, the overall mean of all six patients is highlighted by a thick solid line. Figure 5.4 illustrates that, after topical application of ALA, there is a wide variability seen in the collected PpIX fluorescence signals among the six patients examined at each time point of treatment. Each baseline fluorescence spectrum measurement was subtracted from their corresponding fluorescence spectrum measurement at the three different time points of treatment, i.e., before, half-way through and after treatment.
Figure 5.4 PpIX fluorescence intensity – $F_{PpIX}$ – spectra recorded for six patients and the mean is depicted in bold, (a) immediately prior to PDT treatment; (b) half-way through treatment; and (c) immediately after PDT treatment.

5.3.2 Photobleaching Observed During Clinical Topical Photodynamic Therapy

*In vivo* photobleaching was monitored through the use of the clinical patient sBCC fluorescence data collected and discussed above. Figure 5.5 (a) depicts the mean PpIX fluorescence intensity from the six patients at the time points of treatment. As
expected, this decreases from the outset to the end of treatment, and illustrates how the peak PpIX fluorescence reduces as treatment progresses and photobleaching occurs. For each patient, the PpIX fluorescence intensity values recorded around 635 nm were normalised to each of their individual maximum PpIX fluorescence intensity value. Each point, therefore, in Figure 5.5 (b) represents normalised PpIX fluorescence intensity values recorded around 635 nm from patients at particular time points during their respective treatment times. Assuming photobleaching follows a single exponential decay curve, a time constant, $\tau_{PB}$, of 172 s represents the best fit to the data presented in this figure. The photobleaching dose constant, $\beta$, in J/cm$^2$ was then calculated according to Equation 5.1. At a mean surface irradiance, $(I)$, of 82 mW/cm$^2$, a mean value of $\beta = 14$ J/cm$^2$ with an associated standard deviation of 1 J/cm$^2$ was obtained.

$$\beta = \tau_{PB} \times (I) \quad (5.1)$$

In the literature a range of values of $\beta$ has been provided (1.8 J/cm$^2$ – 33 J/cm$^2$) in various studies [5,6,17,19]. Such a wide variation in the value of $\beta$ may arise from a range of factors including the time between administration of the drug to treatment beginning, the fluence rate used for illumination, the type of animal system investigated and the type of tumour being treated. In our case this value was derived from clinically obtained data for human patients presenting with sBCC.
Figure 5.5 (a) Mean PpIX fluorescence intensity (peak points ~ 635 nm) recorded from six sBCC before, half-way during and after PDT treatment; (b) Normalised PpIX fluorescence intensity (peak points ~ 635 nm) of six sBCC before, half-way during and after PDT treatment.
5.3.3 Monte Carlo Predictions of PpIX Fluorescence Originating in a Tumour

As illustrated in Figure 5.6 (a), PpIX fluorescence photons generated at deeper tumour depths have a lower probability of escape from the tumour than PpIX fluorescence photons generated at shallower tumour depths. When the treatment simulation progressed and photobleaching increased, the PpIX fluorescence emitted from the surface of the tumour decreased and more PpIX fluorescence that originated from deeper within the tumour was observed at the surface. Initially, fluorescence detected at the surface decreased rapidly with depth. As treatment progressed, the fluorescence detected from photons near the surface decreased. From half-way through treatment (75 J/cm\(^2\)) almost all surface fluorescence was derived from photons deep within the tumour. The output power of PpIX fluorescence detected at the tumour surface at LD = 0 J/cm\(^2\), was denoted as \(P_{F_{t=0}} = 6.80 \times 10^{-5}\) W. The total fraction of PpIX fluorescence detected at the tumour surface at LD = 37.5, 75, 112.5 and 150 J/cm\(^2\) compared to \(P_{F_{t=0}}\) was 1.95 x 10\(^{-3}\), 1.20 x 10\(^{-4}\), 2.77 x 10\(^{-5}\) and 3.00 x 10\(^{-6}\) respectively. Figure 5.6 (b), illustrates the effect of increasing LD and photobleaching in the treatment simulation demonstrating a reduction in the total PpIX fluorescence detected at the tumour surface. There is a difference of almost six orders of magnitude in the PpIX fluorescence detected from the surface of the tumour between LD = 0 J/cm\(^2\) (t = 0 seconds) and LD = 150 J/cm\(^2\) (≈ t = 1800 seconds). A decrease of approximately three orders of magnitude is evident between 0 J/cm\(^2\) and 37.5 J/cm\(^2\) indicating a large decrease in PpIX fluorescence pertaining to rapid photobleaching followed by a slower photobleaching thereafter.
Figure 5.6 (a) PpIX fluorescence detected at the surface that has originated from varying depths in the tumour with increasing LD in the treatment and fluorescence simulations; (b) Total PpIX fluorescence detected at the surface of the tumour with increasing LD in the treatment and fluorescence simulations.
In order to compare our model, which used clinical data reported in the present chapter, to clinical data reported by Cottrell et al. [20] results were re-analysed using excitation and emission wavelengths of 632 nm and 705 nm, respectively (Figure 5.7 (a)). Our simulated normalized PpIX fluorescence curve – which corresponds to 82 mW/cm$^2$ – was compared to two other fluence rates, namely, 150 mW/cm$^2$ and 60 mW/cm$^2$ taken from the clinical data for sBCC reported by Cottrell et al. [20]. In the MC model, we used our clinically determined photobleaching dose constant, $\beta$, of 14 J/cm$^2$ and found that our simulated PpIX fluorescence data was in close agreement with the clinical data of Cottrell et al. [20] up to a fluence of approximately 8 J/cm$^2$. Our simulated normalized PpIX fluorescence signal then decreased below the clinical normalized PpIX fluorescence signal corresponding to 60 mW/cm$^2$.

Figure 5.7 (b) is an extension of Figure 5.7 (a) and illustrates how the normalized PpIX fluorescence continues to decrease up to 150 J/cm$^2$. At this point we found that a small quantity of PpIX fluorescence was still evident in the tumour. The data in both these graphs use logarithmic y-axis to display the normalized PpIX fluorescence more clearly. Interestingly, Cottrell et al. [20] illustrated that PpIX fluorescence was still present up to a fluence of 200 J/cm$^2$, when treated with 150 mW/cm$^2$ suggesting that photobleaching may still be occurring.
Figure 5.7 (a) Comparison of normalized PpIX fluorescence (705 nm) detected at the surface of the tumour as a function of treatment light dose (J/cm$^2$) from our model using 82 mW/cm$^2$ to clinical data reported by Cottrell et al. [20] at 60 mW/cm$^2$ and 150 mW/cm$^2$; (b) Total normalized PpIX fluorescence (705 nm) detected at the surface of the tumour with increasing LD in the treatment and fluorescence simulations.
5.3.4 Monte Carlo Simulations of Singlet Oxygen Produced in a Tumour

In addition to the PpIX fluorescence detected, singlet oxygen produced at depths in the tumour was also modeled at each time step. At LD = 0 J/cm² (t = 0 seconds) - in other words before photobleaching – a large quantity of singlet oxygen was evident, particularly at the surface of the tumour. As treatment progressed the superficial layers of the tumour became photobleached and less singlet oxygen was produced in these layers. When the treatment continued further a larger quantity of singlet oxygen could be seen to be produced deeper within the tumour than at the surface. This became more apparent during the latter stages of the treatment simulation. The majority of the PD occurs early on in PDT treatments and reduces as the treatment progresses. However, there is still a small but potentially useful quantity of singlet oxygen produced with an increasing LD in deeper layers of the tumour as shown in Table 5.2. This singlet oxygen produced is expressed as a fraction of the maximum singlet oxygen produced at the surface of the tumour, z = 0, and at the treatment light dose, LD = 0 J/cm², denoted as, $^8$O$_{LD=0,z=0}$ and equal to $5.79 \times 10^{17}$ $1^2O_2/cm^3$.

Table 5.2 Singlet oxygen molecules generated at varying depths in the tumour at specific time points in the treatment simulation as a fraction of the maximum singlet oxygen generated at the tumour surface. ($^8$O$_{LD=0,z=0} = 5.79 \times 10^{17}$ $1^2O_2/cm^3$).

<table>
<thead>
<tr>
<th>Tumour Depth (mm)</th>
<th>$^8$O$<em>{LD,z}$/$^8$O$</em>{LD=0,z=0}$</th>
<th>Treatment Light Dose (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.5</td>
<td>75</td>
<td>112.5</td>
</tr>
<tr>
<td>1</td>
<td>$1.10 \times 10^{-2}$</td>
<td>$2.00 \times 10^{-4}$</td>
</tr>
<tr>
<td>2</td>
<td>$5.50 \times 10^{-2}$</td>
<td>$1.30 \times 10^{-2}$</td>
</tr>
<tr>
<td>3</td>
<td>$5.00 \times 10^{-2}$</td>
<td>$3.00 \times 10^{-2}$</td>
</tr>
<tr>
<td>4</td>
<td>$3.10 \times 10^{-2}$</td>
<td>$2.50 \times 10^{-2}$</td>
</tr>
</tbody>
</table>
The singlet oxygen produced at all time points of the treatment simulation was then added together to obtain the singlet oxygen produced in a tumour over the entire treatment simulation as a function of tumour depth and LD. This is shown in Figure 5.8 (a). The horizontal line indicates the necrosis threshold photodynamic dose, PD_T, assumed to be $8.60 \times 10^{17} \frac{\text{O}_2}{\text{cm}^2}$ absorbed by the photosensitiser per unit volume of tissue [21]. Therefore, we found that the maximum depth achieved of singlet oxygen produced in the tumour after administering a total treatment light dose (LD) of 37.5, 75, 112.5 and 150 J/cm$^2$ was 2.00 mm, 2.70 mm, 3.00 mm and 3.30 mm respectively. This is illustrated in Figure 5.8 (b). PDT with red light is an effective treatment for non-melanoma skin cancers with a thickness of 1 – 3 mm. The results here are in good agreement with those quoted in the literature [1,22].
Figure 5.8 Singlet oxygen production in units of $^{1}O_2$/cm$^3$ generated by the photosensitiser in the tumour, (a) as a function of depth and LD; (b) maximum depth achieved after a given time. The threshold photodynamic dose, $PD_T$, assumed to be $8.60 \times 10^{17} \, ^{1}O_2$/cm$^3$ generated by the photosensitiser is signified by a horizontal line.
5.3.5 Effects of Optical Properties

The results presented in Figures 5.6, 5.7 and 5.8 are based on optical properties described by Salomatina et al.[18], derived from non-melanoma human skin cancer in vitro. These optical properties appear to be well suited for the present study and so were incorporated in the MC model. Further simulations were performed to investigate how the collected PpIX fluorescence signal and the generated singlet oxygen changed with different optical properties. These optical properties – used to represent tumour optical properties – were derived from human skin tissue in vitro, sourced from two independent studies and cover a wide spectral range, which is of interest to us [23,24]. Figure 5.2 (a) and (b) depict the curves of the optical properties used to produce Figure 5.8. The scattering anisotropy factor, g, was taken as 0.8 when using the optical properties derived by Salomatina et al.[18] and 0.9 when using the optical properties derived by both Bashkatov et al.[23] and Chan et al.[24]. Bashkatov et al.[23] had the lowest absorption coefficients and highest scattering coefficients as a function of wavelength for the three optical property sets. Furthermore, Chan et al.[24] had the highest absorption coefficients as a function of wavelength. Figure 5.9 (a) illustrates how different sets of optical properties affected the absolute value of the collected PpIX fluorescence signal, while Figure 5.9 (b) and 5.9 (c) represent their effect on the generated singlet oxygen [23,24]. The photobleaching dose constant, $\beta$ was fixed at 14 J/cm$^2$ for all the simulations when considering different sets of optical properties as inputs for the model. In light of this, optical property variations could explain the differences in the empirical PpIX fluorescence spectra recorded from the 6 patients presented in Figure 5.4.
Figure 5.9 (a) illustrates how different sets of optical properties affected the absolute value of the collected PpIX fluorescence signal; (b) represents the effects of optical properties obtained from Bashkatov et al. [23] on the singlet oxygen production; and (c) represents the effects of optical properties obtained from Chan et al. [24] on the singlet oxygen production.
5.4 Discussion and Conclusion

We employed the PDT implicit dosimetry model, which uses fluorescence photobleaching kinetics of a photosensitiser as a dose metric [2]. Non-invasive monitoring of the in vivo PpIX fluorescence signal collected during clinical PDT enabled the prediction of singlet oxygen generation from which the PDD in a tumour can be inferred. The empirical data showed a reduction in the detected PpIX fluorescence that was used as an indicator of in vivo PpIX photobleaching. From our clinical results an in vivo photobleaching dose constant, \( \beta \), of ALA-induced PpIX fluorescence was found to be \( 14 \pm 1 \text{ J/cm}^2 \). We have shown that the majority of the PpIX fluorescence signal has disappeared half-way during treatment and no longer visible to the naked eye and is again further reduced albeit to a lesser extent at the end of treatment. Similar clinical findings have been reported by Hewett et al. [25], where they point out that there was a discernible reduction in PpIX fluorescence after only 100 seconds (dose = 12 J/cm\(^2\)). Furthermore, they state that the 630 nm fluorescence was reduced to the surrounding tissue background level by 300 seconds into the treatment. Ericson et al. [19], reported a high rate of photobleaching up to a cumulative light dose of 10 J/cm\(^2\).

Our 3D MCRT model provides a qualitative description of both the collected PpIX fluorescence signal and the generated singlet oxygen. MCRT simulations taking into account photobleaching were performed in an attempt to determine the spatial and temporal changes to the origin of collected PpIX fluorescence and generated singlet oxygen with increasing LD and at varying depths in the tumour. This MCRT model has been designed to mimic both the clinical situation under investigation and the procedure by which treatment and in situ monitoring take place. It is capable of simulating entire PpIX fluorescence spectra and enabled us to examine further clinical observations and investigate the inherent, complex nature of PDT. Following a similar route to those present in the literature, a range of assumptions have been made in Section 5.2.4 to enable our investigation of the PpIX fluorescence detected and the singlet oxygen generated during a PDT treatment.

During PDT treatment, the photosensitiser near the surface photobleaches and therefore the PpIX fluorescence detected at greater depths contributes more to the surface fluorescence signals detected [Figure 5.6 (a)]. Figure 5.6 (b) illustrates that most of the surface PpIX fluorescence has diminished towards the end of treatment.
The total fraction of PpIX fluorescence detected at the tumour surface at LD = 37.5, 75, 112.5 and 150 J/cm\(^2\) compared to \(^{5}F_{0}\) was 1.95 x 10^{-3}, 1.20 x 10^{-4}, 2.77 x 10^{-5} and 3.00 x 10^{-6}, respectively. Nonetheless, a small but potentially useful quantity of singlet oxygen was still being produced at depth within the tumour as shown in Table 5.2. A potential limitation to the model is the fact that oxygen concentration is constant and it is assumed that availability of oxygen does not compromise the effectiveness of treatment. It has been reported that oxygenation may be maintained during light illumination, particularly if low fluence rates are used [26]. However, recent studies have reported that \( \beta \) is not constant and is varying both temporally and spatially within the tissue [20,27]. These studies observed fluence rate dependent photobleaching kinetics that were attributable to oxygen supply to the treated tissue. This is particularly true when using different fluence rates.

We acknowledge from Figure 5.7 (a) that in order to reproduce the clinical data of Cottrell et al. [20] more accurately, \( \beta \) would need to increase with time – essentially slowing the photobleaching rate as a function of time and fluence – which is presumably due to decreased oxygen levels as the treatment progresses. This suggests that \( \beta \) may not be constant for a constant fluence rate. However, it is difficult to ascertain how \( \beta \) would change after 12 J/cm\(^2\). Therefore in order to change \( \beta \) accurately as a function of fluence, it is necessary to have comprehensive clinical data, with a large sample size of patients, which extends up to at least 150 J/cm\(^2\). Moreover, in our model we have sought to represent the situation that pertains within most PDT clinics, i.e., excitation at 400 nm for fluorescence induction and treatment at 632 nm. However, Cottrell et al. [20] used different parameters. More detailed studies of fluorescence and/or oxygen measurements \textit{in vivo} are necessary to ascertain how oxygen changes as a function of fluence during treatment. Tumour oxygenation and the change thereof during PDT with systemically administered Photofrin has been studied by Henderson et al. [28] However, the lack of well established real time oxygen measurements recorded \textit{in vivo} during ALA-PDT of sBCC makes it difficult to ascertain how oxygen changes during treatment. Furthermore, the determination of \( \beta \) could be further complicated \textit{in vivo} by variations in the vascularization of the tumour, skin temperature, tissue optical properties and the spatial distribution of the photosensitiser. We have used a constant
\( \beta \) because this fits the clinical data, which was derived in this study. There is certainly a need for more clinical PpIX fluorescence data like that published by Cottrell et al. [20] and our results highlight this. In line with previous work carried out by Farrell et al. [6] we assumed a constant \( \beta \) implying that the tumour was fully oxygenated for the duration of the treatment, which had no limiting effects on the production of singlet oxygen.

Data from clinical ALA-PDT treatments reported by our own group in tumour tissue [29] show a similar time course to that reported by Cottrell et al. [20] namely, a rapid initial photobleaching followed by a slowly decaying fluorescence level as treatments progressed. The slower rate of photobleaching could be due to oxygen as mentioned above and/or due to a spatially inhomogeneous PpIX concentration in the tumour as suggested by Kruijt et al. [30] in rat esophagus.

However, it has been shown that photobleaching in vivo can be depth dependent; as the photobleaching at the surface is completed, the fluorescence decays at a slower rate due to detection of fluorescence emission from deeper layers [16]. It has been reported by other groups that ALA penetration depths may range between 2 and 5 mm [31-33]. We have therefore assumed a homogeneous distribution of PpIX in our modeled tumour, with a thickness of 4 mm, which is within this reported range. We acknowledge that the depth-dependent concentration of PpIX together with delivered fluence is important in PDT dosimetry. It has been reported in the past that increasing the uptake of ALA penetration by deep BCC lesions could be improved by prolonging the topical application time [34]. This could potentially allow for a more homogeneous distribution of PpIX within the tumour. We have sought to investigate the issue of delivered fluence to the tumour. Therefore, we believe that an opportunity exists to optimize PDT regimes for deep BCC by delivering a larger treatment light dose to the tumour.

Monitoring the change in the surface photosensitiser fluorescence signals during PDT due to photobleaching may be used to predict the depth of necrosis [6,35]. The PDT is illustrated in Figure 5.8 by the horizontal lines. Therefore, the depth of necrosis based on the generation of singlet oxygen in the tumour was found to be 2.00 mm, 2.70 mm, 3.00 mm and 3.30 mm after an administered LD of 37.5, 75, 112.5 and 150 J/cm\(^2\) [Figure 5.8 (b)], for an adopted specific set of optical properties. To the best of our knowledge, the optical properties we have used in Figure 5.8 are
the most comprehensive to date representing nonmelanoma skin cancers. These results suggest that an increase from our typical administered treatment light dose of 75 J/cm$^2$ to 150 J/cm$^2$ could increase the effective PDT treatment initially achieved at a depth of 2.7 mm to 3.3 mm in the tumour, respectively. Furthermore, this increase reduced the surface PpIX fluorescence from $1.2 \times 10^{-4}$ to $3 \times 10^{-6}$ of $F_{t=0}$. Oseroff et al. [36], previously suggested the need for a treatment light dose of at least 100 J/cm$^2$ at 635 nm.

It is well known that the effect of tissue optical properties can affect the collected fluorescence signal [37]. Therefore, we need to be cautious in our interpretation of the empirical fluorescence data. Optical property effects may be responsible for the observed variations in the collected PpIX fluorescence signals between the patients presented in Figure 5.4. Correct interpretation of the photobleaching data (Figure 5.5) requires that the empirical PpIX fluorescence data have been corrected for changes in optical properties. MCRT simulations were performed using a range of optical properties from the literature. The results presented in Figure 5.9 indicate that changing the optical properties affected the absolute value of the collected PpIX fluorescence signal and the generated singlet oxygen. This, in turn, impacted on the penetration of light and hence the PDD administered to the tumour. However, the recommendation of administrating a larger light dose, which advocates an increase in the treatment time after surface PpIX fluorescence has diminished, remained valid for different sets of optical properties and therefore should have a beneficial outcome on the total treatment effect.

This MCRT model can be considered as a reasonable approach to establishing tailored optimal treatment regimes for clinical PDT based on in vivo PpIX fluorescence measurements recorded from patients presenting with sBCC. We have demonstrated a relationship between surface PpIX fluorescence and the photodynamic dose, PDD, at varying depths in a tumour and at different LD administered during a simulated PDT treatment, in the presence of photobleaching. In light of the results presented in this chapter, we suggest that an increase in the treatment light dose beyond the disappearance of surface PpIX fluorescence may continue to provide effective PDT treatment at depth within tumours. This increase in the time of light administration may ultimately assist in optimizing PDT treatment regimes. If patients were treated for longer, this could potentially eliminate remnants of the lesion deeper
down in the skin tissue eradicating residual disease and reducing recurrence rates. Administrating a larger treatment light dose means increasing the treatment time and this may have a negative impact on patients, particularly those who experience severe pain during treatment. Pain can be a limiting factor to successful PDT [38]. However, it has been shown that PDT pain is higher initially and decreases during treatment [19]. Failure to deliver effective PDT treatments, impact the welfare of patients and their quality of life. Follow-up in PDT clinics are critically important and decisions are facilitated pending the outcome of these assessments. Ultimately, if longer treatment times were advocated, it may be possible to achieve highly successful long-term clinical outcomes, while saving time, money and hospital resources.
5.5 References


Chapter 5 – Monte Carlo Radiation Transfer Modelling in Topical PDT


Chapter 6 – Light Distributions in Topical Photodynamic Therapy

6.1 Introduction
The deep penetration of light into tissue is an important aspect required for PDT. When treating tumours, the maximum penetration depth may be obtained by choosing the correct wavelength of light [1], since $\mu_t$ is wavelength dependent. Since PDT is a three-part therapy that relies on the interaction of light photons with photosensitiser molecules and oxygen, photons need to impinge their energy sufficiently to the existing photosensitiser in the target region of interest. The choice of light source is important for the efficacy of PDT treatments [2]. The characteristics of any chosen light source, laser or non-laser, will have an impact on PDT dosimetry. As light is an important element in any PDT treatment, it is of interest to investigate the distribution of light as it propagates through tumour tissue. The preferred light sources in PDT are those, which achieve wide illumination of large areas that is often required for AK and BD [3]. This serves two purposes; firstly, to accomplish the deepest penetration of light and secondly the potential to treat large lesions.

In the present chapter, non-laser light sources each with their own spectral emission were compared in terms of the depth of light penetration achievable in the tumour and the PDD administered to the tumour, by means of a MCRT model, previously described in Chapter 4. The path of monochromatic light through a tumour was simulated by the MCRT model. Five different wavelengths were investigated, namely, 405 nm, 505 nm, 540 nm, 575 nm and 630 nm, each of which correspond to the absorption peaks associated with the characteristic PpIX absorption spectrum. Light penetration, absorption and consequently the dose deposition in the tumour were compared at each wavelength with particular attention focusing on the extreme wavelengths of 405 nm and 630 nm. Final comparisons were drawn between all five wavelengths with emphasis on the PDD in the tumour after treatment, i.e., 75 J/cm². As discussed previously in Chapter 5, we construct a model of a tumour of finite size embedded and surrounded in normal tissue was subjected to a finite width uniform superficial irradiation. A comparison of light propagation through a tumour using finite beam diameters was undertaken. The light distribution profiles were again

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simulated using the MCRT model. Moreover, the PDD deposition pertaining to each beam diameter was computed by this technique at depth in the tumour. Also, the propagation of light into deep-seated tumours was assessed by means of the MCRT model. Starting from an original superficial position in the cube grid, the modelled tumour was moved progressively deeper, analogous to the way that tumours may be located in normal tissue. The affect of re-positioning the tumour and the impact thereof on the PDD is identified and discussed accordingly.

Light-tissue interactions and the light distribution within the tissue depend on both the characteristics of the light and the optical properties of the tissue [4]. The optical penetration depth describes the optical transparency of tissue and is defined as the depth in the tissue at which the intensity of the propagating light falls to approximately 37 % (1/e) of the incident value [5]. This will evidently have an impact on the clinical effectiveness of PDT treatments. To the best of our knowledge, the difference between monochromatic and broadband light sources and light delivery has not been compared in the published literature. The manner in which light exhibiting different characteristics propagates a modelled tumour together with both the light dose and photodynamic dose administered will be presented in this chapter, along with a detailed analysis and description of the findings from the MCRT dose model. The optical properties previously used in Chapter 5 – those published by Salomatina et al. [6] – were again adopted for all the simulations performed in this chapter.

6.2 Light Sources in PDT
There exist a number of light sources – lasers and non-coherent sources – which enable PDT to be routinely used in hospital clinics [7,8]. A light source is described by its spectral bandwidth and the choice of light source used in PDT may be determined according to the location of a tumour. Lasers are the best equipped for internal use as it is necessary for the light to be delivered via an optical fibre at adequate power to almost every site of the body, including PDT treatments of the brain. PDT is currently performed at Ninewells hospital to assist in neurosurgery operations. These coherent light sources may be used for PDT due to their ability to serve as monochromatic light sources. The single output wavelength that they offer can selectively target and efficiently excite photosensitisers, such as porphyrins [9]. As shown in Figure 6.1, non-laser light sources, such as lamps and LEDs, have been
exercised for PDT of dermatological conditions, i.e., skin lesions as light may be delivered over a broad spectrum directly to the tumour and without the need to transmit light via an optical fibre [10,11]. The application of light directly onto the surface of skin lesions has placed lasers and non-laser light sources on par for PDT treatments. As light delivery is an important aspect of light irradiation in PDT, it is essential to achieve sufficient power in the wavelength range corresponding to the photosensitiser absorption [12]. Figure 6.2(a) depicts the normalised spectral emission measured from four different non-laser light sources, which are commonplace at PDT treatment centres [13]. Each light source may be employed in topical ALA- and MAL-PDT, as their maximum peaks are centred on approximately 630 nm, which corresponds to the last Q-band – Chapter 1, Section 1.2, Figure 1.3 – in the PpIX absorption spectrum, enabling absorption to occur at the deepest possible level in the tumour. Tissue absorption and scattering characteristics must be considered when investigating light penetration and distribution through a tumour in an effort to optimise treatment parameters. The tungsten filament quartz halogen lamp (Photocure) and the metal halide lamp (Waldmann 1200) illustrated in Figure 6.2(a) produce a broadband spectral emission. Also, the xenon arc lamp (Paterson) emits broad spectrum radiation which can be filtered. These light sources have been employed in PDT of superficial lesions such as nonmelanoma skin cancer [14]. A LED array known as the Aktilite produces a spectral emission with a narrow bandwidth in the region of 5 – 10 nm and is currently the preferred light source for PDT treatments carried out at Ninewells Hospital, Dundee [15]. Figure 6.2(b) compares the four different light sources in terms of spectral irradiance (mW/cm²/nm) at the skin surface. Typical exposure times required to attain a treatment light dose of 75 J/cm² vary from lamp to lamp. For instance, for broad-spectrum light a surface irradiance of 100-250 mW/cm² is normally chosen – depending on the filters used – which administers a light dose of 75 J/cm² in approximately twelve to five minutes, while light emitting diodes (LED) with a narrow spectral emission have values in the range of 70-100 mW/cm², which administers a treatment light dose of 75 J/cm² in approximately eighteen to twelve minutes [7, 15]. However a treatment light dose of 100-150 J/cm² is normally chosen for broad-spectrum light [3].
Chapter 6 – Light Distributions in Topical Photodynamic Therapy

Figure 6.1 Four light sources that have been used for PDT treatment of skin lesions.

(a) Quartz Halogen Lamp (Photocure)  
(b) Xenon Arc Lamp (Paterson)  
(c) Metal Halide Lamp (Waldmann 1200)  
(d) LED (Aktilite)
Figure 6.2 (a) Normalised Spectral Emission from four different light sources employed in PDT treatment of skin lesions; (b) Comparison of the four different light sources in terms of the spectral irradiance at the skin surface.
6.3 MCRT Modelled Light Sources

The four different light sources mentioned above were used as inputs into the MCRT model. Simulations were performed investigating the impact each light source had on the depth of light penetration through a modelled tumour and the PDD administered to it. For each simulation the tumour was exposed to a constant surface irradiance of 82 mW/cm². These simulations were designed to examine which light source was the most efficient for PDT treatment. The results from the model suggest that the Paterson lamp and LED (Aktilite) were capable of delivering light to the deepest layers of the tumour. This, in turn, facilitated in depositing a greater PDD throughout the entire tumour volume over the course of a specified simulation treatment time of 900 seconds or equivalently a light dose of 75 J/cm².

Figure 6.3 Singlet oxygen production rate – $^{1}O_2$ cm⁻³ s⁻¹ – at 2 J/cm² in the treatment simulation (a) Photocure; (b) Paterson; (c) Waldmann 1200; and (d) Aktilite.
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Figure 6.4 Singlet oxygen production rate – $^{1}O_2$ cm$^{-3}$ s$^{-1}$ – at 75 J/cm$^2$ in the treatment simulation (a) Photocure; (b) Paterson; (c) Waldmann 1200; and (d) Aktilite. The late stage of the treatment – 75 J/cm$^2$ – is represented here, illustrating the variation of photosensitiser concentration at depth. As the top layers of the tumour have become photobleached during treatment, there is a higher singlet oxygen production rate at depth at 75 J/cm$^2$.

The snapshots depicted above in Figure 6.3 and Figure 6.4 relating to each of the four light sources enabled the light penetration into the tumour to be examined. These profiles are representative of absorption in the tumour at the beginning of the treatment simulation, i.e., 2 J/cm$^2$ and at the end of the treatment simulation, i.e., 75 J/cm$^2$. For further analysis, the values pertaining to the PDD at the surface, 1 mm, 2 mm, 3 mm and 4 mm at depth in the tumour were tabulated for 2 J/cm$^2$. The values shown in Table 6.1 are typical of the PDD administered to the tumour at a specific light dose in the treatment simulations for each light source as a function of tumour depth. These demonstrate that (b) Paterson and (d) Aktilite are comparable, while showing an appreciable difference to (a) Photocure and (c) Waldmann 1200. The cumulative PDD administered to the tumour was plotted as a function of depth over the entire treatment simulation with depictions after 2, 18, 37.5 and 75 J/cm$^2$ reported.
Figure 6.5 illustrates that (a) and (c) are less effective for PDT treatment of skin lesions with 82 mW/cm\(^2\). In these instances, the threshold PDD is not exceeded in the PDT dose model, implying that the use of these light sources at this particular surface irradiance is insufficient. Meanwhile, the lamps of narrow bandwidth (b) and (d) are more appropriate for eliciting a PDD greater than the threshold PDD to a tumour depth of 2.75 mm and 2.70 mm, respectively when a surface irradiance of 82 mW/cm\(^2\) is employed. Lamps providing a broadband spectral emission cover a larger range of wavelengths, thereby reducing the surface irradiance spread out over the wavelengths. A broad bandwidth can lead to a decrease of the PDD effect as shown in Figure 6.5. Alternatively, narrow bandwidths enable the chosen surface irradiance to be maintained close to the targeted wavelength of interest. Increasing the quantity of photosensitiser administered to the tumour could assist in overcoming the lack of efficiency presented by the broadband light sources. Interestingly, the model outputs indicated that (b) Paterson, compared to the other three lamps administered a threshold photodynamic dose (PD\(_T\)) to the greatest depth within the tumour, albeit a very small increase over the Aktilite.

**Table 6.1** The singlet oxygen production rate – \( ^1O_2 \text{cm}^{-3} \text{s}^{-1} \) – at 2 J/cm\(^2\) in the treatment simulations for each light source as a function of tumour depth.

<table>
<thead>
<tr>
<th>Tumour Depth (mm)</th>
<th>(a) Photocure</th>
<th>(b) Paterson</th>
<th>(c) Waldmann</th>
<th>(d) Aktilite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Dose = 2 J/cm(^2)</td>
<td>PDD ((^1O_2)/cm(^3))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>3.08 x 10(^{17})</td>
<td>5.98 x 10(^{17})</td>
<td>2.27 x 10(^{17})</td>
<td>5.73 x 10(^{17})</td>
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<td>1.62 x 10(^{17})</td>
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<td>1.30 x 10(^{17})</td>
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</tr>
<tr>
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<td>2.43 x 10(^{16})</td>
<td>8.13 x 10(^{15})</td>
<td>2.32 x 10(^{16})</td>
</tr>
</tbody>
</table>
Chapter 6 – Light Distributions in Topical Photodynamic Therapy

Figure 6.5 PDD in units of \( ^1O_2/cm^3 \) generated by the photosensitiser in a modelled tumour as a function of depth using four light sources (a) Photocure; (b) Paterson; (c) Waldmann 1200; and (d) Aktlite. The threshold photodynamic dose, \( PD_T \), assumed to be \( 8.60 \times 10^1 O_2/cm^3 \) generated by the photosensitiser is signified by the horizontal line.

6.4 Monochromatic Light Sources

PDT may be performed at any wavelength where there is the potential of absorption. This light is capable of activating the photosensitiser and eliciting cytotoxic effects to the tumour [16]. The smaller the bandwidth, the more truly monochromatic the light source is. The wavelength of the chosen light source influences the degree of scattering and absorption present in the tumour. Figure 6.6 demonstrates quite clearly the different depths attainable by wavelengths of light important in PDT [17]. Shorter wavelengths, such as blue light (405 nm) are greatly scattered and absorbed so that they have a limited penetration of only 1 – 2 mm into the skin [18]. However, longer wavelengths corresponding to red light (630 nm) and infra-red light (1064 nm) are scattered and mainly absorbed to a lesser extent and therefore may penetrate tissue more deeply and effectively. It has been reported that light in the wavelength range of 400 – 500 nm has a 50 – 200 % reduced tissue penetration when compared to light in
the spectral range of 600 – 700 nm [19,20]. Moreover, according to the American Association of Physicists in Medicine (AAPM), PDT may result in therapeutic zones of approximately 11 mm in depth [21].

Focusing on monochromatic light has several implications for PDT treatments. PpIX is an efficient photosensitiser, which exhibits a Soret band – the most intense absorption band at approximately 405 nm – and four smaller Q bands at 505 nm, 540 nm, 575 nm and 630 nm. Although absorption is greater at 405 nm there is an associated issue of limited penetration and distribution of light at depth in the tumour volume. Figure 1.3 illustrates the characteristic PpIX absorption spectrum with the 5 distinctive absorption bands and the related excitation wavelengths, which activate the photosensitiser. Figure 6.7 depicts the propagation of light and absorption profiles through the modelled tumour for each wavelength. The profiles are snapshots recorded at the beginning of the treatment simulation, approximately 2 J/cm². Depths of 2 mm, 3 mm and 4 mm in the tumour were compared and analysed in order to sample and report the light photons that could propagate to the deeper regions of the tumour. From Table 6.2 it is shown that a larger quantity of photons is absorbed in the 2 – 4 mm region of the tumour when employing the 630 nm instead of the 405 nm.

**Figure 6.6 Depth of light penetration in skin tissue for PDT related wavelengths [17].**
Comparing these wavelengths emphasise that 630 nm absorption is greater, and by a factor of 4.95, 32 and 108 at 2 mm, 3 mm and 4 mm, respectively. In agreement with the literature, these graphs show the superior penetration characteristics of the 630 nm wavelength over the other test wavelengths [5]. Consequently, a more effective PDD would be distributed to the whole tumour volume, which in turn would elicit a greater clinical effective PDT treatment.

**Figure 6.7** The singlet oxygen production rate – $^{1}O_2$ cm$^{-3}$ s$^{-1}$ – at 2 J/cm$^2$ using five different monochromatic wavelengths (a) 405 nm; (b) 505 nm; (c) 540 nm; (d) 575 nm and (e) 630 nm.
Table 6.2 Comparison of the PDD at 2 J/cm² between 405 nm and 630 nm.

<table>
<thead>
<tr>
<th>Tumour Depth (mm)</th>
<th>405 nm</th>
<th>630 nm</th>
<th>Factor Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5.89 x 10¹⁶</td>
<td>2.92 x 10¹⁷</td>
<td>4.95</td>
</tr>
<tr>
<td>3</td>
<td>3.28 x 10¹⁵</td>
<td>1.05 x 10¹⁷</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>4.33 x 10¹⁴</td>
<td>4.68 x 10¹⁶</td>
<td>108</td>
</tr>
</tbody>
</table>

Monte Carlo simulations were carried out and the PDD contours illustrated in Figure 6.8 were compiled, by summing together all the snapshots and comparing the effect of using different monochromatic PDT wavelengths. In line with previous simulations presented in Chapter 5, the modelled tumour was subjected to a surface irradiance of 82 mW/cm². Figure 6.8 (a) – (e) typify three distinct points in the treatment simulation corresponding to 25 %, 50 % and 100 % of the total light dose. Finally, Figure 6.8 (f) is shown for comparison of the five wavelengths at 75 J/cm². The model indicates that the PDD is initially much greater when employing 405 nm compared to any other wavelength – due to increased PpIX absorption – which then decreases rapidly as a function of depth into the tumour and eventually drops below the threshold PDD line at 2.23 mm after 75 J/cm². Conversely, the PDD is the lowest at the outset of treatment with 630 nm but maintains a more steady administration of PDD as a function of tumour depth, which decreases slowly and finally converges at the threshold PDD line at 3.81 mm after 75 J/cm². This is due to the change in the absorption spectrum of PpIX as a function of wavelength, as shown previously in Chapter 1, Section 1.2, Figure 1.3. The three remaining intermediate wavelengths of 505 nm, 540 nm and 575 nm display PDD contours intersecting the PDD threshold line at 2.63 mm, 2.82 mm and 3.44 mm, respectively.

Interestingly, deep penetration of light is not always the main objective. The use of shorter wavelengths may be preferred when targeting organs such as the bladder, as it is important that the underlying and surrounding normal tissue remains undamaged by the assault of the incoming light [22]. These wavelengths aim to reduce the depth of light penetration into the tissue, while localizing the light at the tumour and effectively sparing the normal tissue positioned beneath the tumour. Therefore, to apply a local PDT treatment to specific target regions, the choice of wavelength delivered is important.
Figure 6.8 PDD as a function of depth using five monochromatic wavelengths (nm) (a) 405; (b) 505; (c) 540; (d) 575; (e) 630 and (f) comparison of all five at 75 J/cm².
6.5 Effect of Beam Diameter

The effect of a beam diameter on the propagation of light through tumour tissue will be studied in this section. Light sources play a pivotal role in PDT and parameters associated with these devices may be manipulated in order to maximize the PDT effect. Keijzer et al. [23] have shown – Figure 4.12 – that by increasing the diameter of a light source, it is possible for the light to be distributed deeper within the tissue. The rationale behind this is that the depth of light penetration is dependent on the area of the treated site over which the light is distributed. When a tumour is exposed to a specific surface irradiance, the light distributed within the tissue must be predicted from this irradiance. This would be a difficult and complex task, without implementing radiative transfer models of scattering biological tissue to infer the deposited light dose inside the tissue. For the purposes of the simulations performed in this section, the tumour diameter and thickness were fixed at 10 mm and 4 mm respectively, while the beam diameter was enlarged from 10 mm to 50 mm, incorporating an intermediary value of 20 mm. As before in Chapter 5, the dimensions of the model were based on a cube grid where a tumour was embedded in normal tissue and subjected to a uniform surface illumination.

The power of a light source – mW – in combination with a specific spot size – cm$^2$ – results in a power distributed over a defined area. This power per unit area is known as the power density or the irradiance – mW/cm$^2$ – of the incident beam. Light distributions in tumour tissue with appropriate optical properties found in the literature – Figure 5.2 – for three different beam diameters – of uniform incident surface irradiance; 82 mW/cm$^2$ – were simulated. Each time the beam diameter, i.e., area was increased, the power – or luminosity – was increased concurrently in the model. This ensured a constant irradiance. To investigate the effect of changing beam diameter, the depth of light penetration was recorded from vertical slices (x, z) obtained at the centre of the cube essentially representing snapshots of the treatment and its progression towards 75 J/cm$^2$. Figure 6.9 (a) – (c) presents the light absorbed at varying depths in the tumour associated with each beam diameter. These images are indicative of absorption occurring within the tumour at 2 J/cm$^2$. 

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Figure 6.9 Singlet oxygen production rate – \( ^1\text{O}_2 \text{cm}^{-3}\text{s}^{-1} \) – at 2 J/cm\(^2\) in three treatment simulations with a tumour of fixed diameter/width – 10 mm – and fixed thickness – 4 mm – with beam diameters (a) 10 mm; (b) 20 mm; and (c) 50 mm.

The PDD administered to the tumour as a function of depth, after a cumulative light dose of 75 J/cm\(^2\), was compared for each of the three beam diameters and represented by Figure 6.10. The PD\(_T\) was achieved down to a maximum depth of 3.25 mm when simulating all three beam diameters. For a constant fluence rate of 1 W/cm\(^2\), Keijzer et al. [23] demonstrated – Figure 4.12 – that by increasing the beam diameter, light could be distributed deeper into the tissue. Interestingly, the beam diameters were quite small increasing from 200 \(\mu\)m to 1 mm to 4 mm, which illustrated a fluence rate of 0.1 W/cm\(^2\) – for all beam diameters – at increasing depths of 0.3 mm to 1 mm to 1.3 mm, respectively [23]. The difference in depth gained by each beam diameter increase – Figure 4.12 – is apparent, which decreases from 0.7 mm to 0.3 mm as the beam diameters are increased. In support of this, Carroll and Humphreys [24] have reported that increasing the diameter of a beam from 7 mm to 10 mm is responsible for an increase in the depth of light penetration, which then levels off as the beam diameter is increased further beyond 10 – 12 mm. Therefore, the effect of increasing
the beam diameter is greater at smaller beam diameters. Figure 6.9 and Figure 6.10, which examines the effect of increasing the beam diameter from 10 mm to 50 mm on the light distribution, are consistent with this report.

![Graph showing PDD as a function of tumour depth in four treatment simulations at 75 J/cm² with beam diameters (green) 10 mm; (black) 20 mm; and (red) 50 mm.]

**Figure 6.10** PDD as a function of tumour depth in four treatment simulations at 75 J/cm² with beam diameters (green) 10 mm; (black) 20 mm; and (red) 50 mm.

### 6.6 MCRT Modelling of Deep-Seated Tumours

The propagation of light into deep-seated tumours was also investigated by means of the MCRT model. The previously used modelled tumour with a 4 mm thickness and a 10 mm diameter was moved beneath the surface of the cube and situated at varying depths in the cube grid. The chosen tumour positions were at 0.6 mm, 1 mm, 1.6 mm and 2 mm. Figure 6.11 (a) – (d) below show the theoretically modelled tumours and their central positions in the cube grid. The bordering white areas are indicative of normal skin tissue, which surround the tumour. As expected, the penetration of light decreases as a function of depth into the tumour. By comparing these varying tumour positions against each other it was possible to estimate with the PDT dose model, the
depth below which a tumour would not be effectively treated. Figure 6.12 below demonstrates that if tumours are situated too far beneath the surface, typically in the region of 2 mm, a desirable PDT effect will not be achieved. Table 6.3 lists the positions of the tumour in the cube and the corresponding depths within the 4 mm tumour, which accomplish at least a threshold $PDT$ after the administration of 75 J/cm². Therefore, the MC model findings suggest that tumours positioned at depths, greater than 1 mm will have a large reduction in the $PDT$ administered within the tumour.

An inadequate PDT effect administered to deep-seated tumours could be due to the transmission of the incoming light through the normal skin tissue. The results presented in this chapter are based on optical properties published by Salomatina et al. [6] – Figure 5.2 – which were derived from NMSC in vitro and normal skin tissue. These optical properties are believed to be well suited and therefore representative of the absorption and scattering characteristics of tumour tissue and normal skin. According to Salomatina et al. [6] the reported absorption and scattering coefficients are larger for normal skin than for tumour tissue. As the light traverses a more opaque layer of skin tissue before reaching its target tumour destination, photons are more likely to be scattered and absorbed and in effect penetrate the tumour insufficiently. This indicates that for the treatment of deep-seated tumours, it would be necessary to implant fibres in situ close to the region of interest and treat accordingly. An alternative option would be to increase the photosensitiser dose administered to the tumour, which implies an increase in the PDD.
Figure 6.11 Singlet oxygen production rate $^{1}O_{2}$ cm$^{-3}$ s$^{-1}$ – at 2 J/cm$^2$ in four treatment simulations where the tumour was positioned below the surface (mm) (a) 0.6; (b) 1; (c) 1.6 and (d) 2.
Figure 6.12 PDD as a function of tumour depth – 4 mm – in four treatment simulations at 75 J/cm$^2$ where the tumour was positioned below the surface (mm) (black) surface; (red) 0.6; (green) 1; (navy) 1.6; and (cyan) 2.

Table 6.3 $P_D_T$ achieved at specific tumour depths as a function of tumour position; and the % Reduction in $P_D_T$ @ 2.70 mm for each tumour position.

<table>
<thead>
<tr>
<th>Tumour Position</th>
<th>Tumour Depth</th>
<th>% Reduction in ($P_D_T$) @ 2.70 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>($P_D_T$) @ 2.70 mm</td>
<td>0.00 %</td>
</tr>
<tr>
<td>Depth: 0.6 mm</td>
<td>($P_D_T$) @ 2.00 mm</td>
<td>25.60 %</td>
</tr>
<tr>
<td>Depth: 1.0 mm</td>
<td>($P_D_T$) @ 1.50 mm</td>
<td>44.44 %</td>
</tr>
<tr>
<td>Depth: 1.6 mm</td>
<td>($P_D_T$) @ 0.20 mm</td>
<td>92.59 %</td>
</tr>
</tbody>
</table>
6.7 Conclusion

The principle objective for the light source and delivery device for PDT is to achieve the desired light dose throughout the tumour volume. This involves a combination of factors, such as the total output power at an appropriate wavelength – which activates the photosensitiser for the generation of the cytotoxic photodynamic action – optimum penetration into the tumour and the corresponding spatial distribution of the power delivered to the tumour [25]. The appropriate delivery of light and the photosensitiser within the target volume has a large influence on the clinical effectiveness of PDT [8].

Previously, a concept of total effective fluence, \( E_d \), has been used, in order to compare light sources. This concept combined the incident spectral irradiance, tissue transmission and the absorption properties of the photosensitiser, resulting in an effective fluence at a chosen depth along with a measure of absorbed dose [26]. Determining the light fluence in tumours is quite difficult but it is possible to estimate the dose and PDT effectiveness within tumours based on radiative transfer models of light transport, such as Monte Carlo. The application of a MCRT model to these questions offers a practical insight.

The research presented in this chapter compares the optical transmission of light and the therapeutic efficacy of light sources, choice of wavelengths and light delivery. It has also sought to represent an approach investigating issues associated with treating deep-seated tumours. It is suspected that the PDT dose model outputs offer helpful results and meaningful support to an essential aspect of PDT.

A wide spectral bandwidth can lead to a decrease in the PDD effect as shown in Figure 6.5. Reduced PDT efficacy is evident as there is a large spatial distribution of the power spread out over the tumour area. It should be noted that PDT with broadband light sources is effective. However, there is a lack of knowledge pertaining to accurate PpIX concentrations in tumours, which are necessary for the PDT effect. The narrow spectral light sources together with the PpIX concentration chosen in this study – which was within those quoted in the literature [27] – illustrated that the PD\(_T\) could be achieved at various depths in the tumour, upon the prescription of specific treatment light doses. This was not the case when the broadband light sources were used. In relation to our model, the PDD is directly related to the photosensitiser concentration and the light fluence rate as described earlier in Chapter 4, Section 4.2,
Equation 4.22. Therefore, the initial PpIX concentration at the beginning of PDT has an important impact on the PpIX concentrations during treatment, which ultimately has an effect on the PD_T.

Figure 6.7 and 6.8 illustrates that the light absorption and consequently the dose deposition as a function of tumour depth, respectively was greatest at 630 nm compared to 405 nm. Increasing the wavelength influences the depth of light penetration [5]. Light of 630 nm has the potential to reach deeper regions within the tumour and therefore enables a larger volume of the tumour to be treated. Increasing the treatment light dose influences the depth of light penetration and consequently the PDD administered at depth in the tumour (Figure 6.5 and Figure 6.8).

A desired depth of light penetration is important when treating various types of tumours. The change in photosensitiser absorption at the excitation wavelengths impacts on the penetration of PDT. Interestingly, a reduced penetration depth may be advantageous when treating for instance an organ such as the bladder [22]. This minimises the potential of unnecessary damage that may be sustained to underlying normal healthy cells, while maximising the selective destruction of tumours. The selection of certain wavelengths could potentially result in less peripheral damage occurring at healthy tissue sites surrounding the tumour.

Undoubtedly the choice of beam diameter is important for clinical PDT. The size of the beam diameter will have an effect on the light distribution. However, this effect is more evident when employing small beam diameters, as shown previously in Figure 4.12.

The optical properties of the target volume may not necessarily be the same as the surrounding tissue optical properties. Therefore, the light propagates with marked differences in absorption and scattering through varying tissue types. This will impose certain restrictions on the depth of treatment and ultimately on the deposited quantity of light energy in the target tissue required for the desired effect. Optical properties derived by Salomatina et al. [6] from NMSC in vitro exhibit varying optical properties between normal and tumour tissue and appear to be well suited to this study. It can be appreciated that the position of tumours – situated closer to the surface – will assist in allowing light to penetrate into deeper regions of the tumour (Figure 6.11 and Figure 6.12). A tumour positioned at 2 mm – Figure 6.12 – away from the uniform surface illumination was not administered an effective PDD, due to inadequate light
illumination to the target volume. A reasonable interpretation of the findings relating to deep seated tumours is the affects of normal tissue optical properties on the propagation of light through the cube grid before and eventually traversing the modelled tumour.

Lasers are coherent, monochromatic and produce a high power output that may be used for the treatment of tumours directly or alternatively be easily coupled to optical fibres, which in effect may allow for PDT to take place in close proximity to the tumour in situ. Lasers have the advantage of providing more specific and selective wavelengths, which maximise transmission of radiation through the tumour and enhance treatment efficiency. Moreover, accurate light dosimetry at the surface of the lesion can be achieved. However, lasers are expensive and maintenance of such devices is carried out routinely. Non-coherent light sources are more widely used than lasers for surface irradiation in PDT because of their wide illumination fields, low cost and portability [2]. For these reasons, lamps play a useful role in PDT. Lamps discharge light over a broad range of wavelengths, which can match varying absorption maxima of several different photosensitisers, potentially resulting in the excitation of those photosensitisers. Lamps are generally used for direct illumination of the tumour and filters are normally required to block out unwanted emission from the light source. There are disadvantages associated with broad emission spectra, for instance, with these light sources the depth of light penetration and the spectral intensity may vary across the bandwidth of the light used [2]. The versatility and portable nature of LEDs together with their narrow 5 – 10 nm bandwidth serve as comprehensive light sources. From a light delivery standpoint, they have the ability to illuminate the tumour directly, deliver a sufficient output power and irradiate the total surface of the tumour. They are compact, cheap and allow the user to continuously monitor the light output and light dosimetry during PDT treatments. Another important aspect of LEDs is that they can be positioned to conform to the shape and size of the tumour presented on the skin by the patient. Furthermore, LEDs have grown in popularity due to their price and ease of use in clinical settings. They are currently the light source of choice for PDT treatments performed at Ninewells Hospital and Medical School.

It is apparent that, when considering a light source for PDT, it is essential to fulfill two main criteria; firstly, a wavelength of light, which matches the absorption
band of the photosensitiser, inducing the desired photochemical reaction and secondly, the deep penetration of the light into the tumour ensuring complete irradiation and consequently treatment of the tumour. Furthermore, consideration should also go into the location and size of the tumour. The distribution of light and the calculation thereof is essential for determining accurate PDT dosimetry, ensuring that adequate light illumination is achieved throughout the target tumour volume. Understanding the light distribution in tissue will significantly play a role in defining and standardising a more precise dosimetry for PDT. The clinical efficacy of PDT depends on the pattern of light delivery, such as, the fluence rate, total treatment light dose and the exposure time [2].

It has been highlighted throughout this chapter that the optimality of PDT treatments is dependent on several factors, such as, light dose, the spectral composition of the irradiance, tissue and photosensitiser absorption and penetration. The MCRT model has been extended to address these issues, in order to infer light propagation and the distribution of the absorbed energy over depths in a tumour pertaining to clinically significant values.
6.8 References


Chapter 7 – Concluding Remarks

7.1 Summary
The research reported in this thesis was performed with topical PDT in mind; a key treatment option used frequently in dermatology mainly for the treatment of NMSC. Although CR are quite high for topical PDT and superior cosmesis is achieved when compared with other therapeutic modalities, it is still however a treatment for selected lesions. As previously mentioned in Chapter 1, Section 1.2, recurrence rates of 20% for BD and sBCC have been reported at 2 years post treatment [1]. Clearly there is a need to improve and extend the role of topical PDT in dermatology for the treatment of NMSC, including more difficult to treat lesions, such as nBCC.

The main motivation for the work undertaken here was to investigate variables in PDT treatment regimes. In an effort to address this key objective, research from an experimental, clinical and theoretical perspective was undertaken. Understanding the synergistic relationship of multiple parameters involved in PDT is vital for successful treatments. In order to improve treatment regimes, it is important to establish optimal treatment times, light doses, choice of light source and irradiance, distribution of light within a tumour, tissue oxygenation, and photosensitiser pro-drug type, concentration and application time. Undertaking clinical studies and mathematical modelling may assist in refining treatment parameters. By combining each of these research areas, a more in-depth understanding of dosimetry can be achieved. This has the potential to directly impact on the success of clinical PDT regimes. This research has sought to gain a deeper understanding of the interaction between the incident light and the tumour. A goal of this thesis was to model both PD and PDT.

In Chapter 2, particular attention was paid to optical diagnostic techniques. The potential advantages of these techniques over histopathology are clearly evident insofar as they offer a real-time, non-invasive, rapid diagnosis with a substantially lower burden on the patient and the health care system. Using a custom designed OBS, specific emphasis was placed upon the application of fluorescence spectroscopy to PDT. Using this spectroscopic tool, Chapter 3 outlined a quantitative comparison of ALA– and MAL–induced PpIX fluorescence, photobleaching and pain recorded during PDT of NMSC. PDT-induced pain, which is a major limiting factor for successful PDT was assessed through the use of a novel pain logger device. This
study offered a quantitative insight into the fluorescence and photobleaching kinetics of PpIX and pain, which in turn, provided pertinent information concerning the choice of optimal photosensitiser pro-drug for PDT. Ultimately, results from this study indicated – on the basis of the presented fluorescence and pain data – that either ALA or MAL may be equally suitable for PDT of NMSC [2].

PpIX fluorescence may be used to identify and delineate lesions from normal surrounding skin, and monitor the build-up and destruction of PpIX during treatment [3]. Consequently, PpIX fluorescence photobleaching during PDT offers an insight into the dosimetry problem involved in treatment regimes. Unquestionably, it is difficult to ascertain exact treatment variables and the fact that each variable may influence the other, complicates PDT dosimetry still further [4]. Dosimetry approaches have been developed, which aim to provide a prediction of tissue damage [5]. An implicit dosimetry model based on a single parameter – fluorescence photobleaching – was used in order to predict \(^1\)O\(_2\) generation, which is assumed to be related to tissue damage. Therefore implicit dosimetry may be incorporated into a treatment protocol, which in turn, can assist in treatment monitoring and planning without the need to measure each treatment variable on an individual basis.

Chapter 4 presented a mathematical model along with an experimental validation and theoretical validations. In an attempt to further investigate treatment variables, this MCRT model, initially developed for astronomy, was adapted and applied to PDT of NMSC. Chapter 5 offered a specific application of this model – which employed the implicit dosimetry approach – to PDT. It was informed by a clinically determined photobleaching dose constant, \(\beta\), established from a data set of clinical results presented in Chapter 3. The purpose here was to gain more of an insight into the pertinent treatment parameters involved in PDT, particular the treatment light dose. Therefore, the MCRT model was employed to address several important clinical PDT questions, so as to further optimise treatment regimes. Specific questions investigated were:

1) How long after surface PpIX fluorescence has diminished is PDT still effective?

2) What depths below the surface is effective treatment provided?

The MCRT findings indicated that an increase from a typical administered treatment light dose of 75 J/cm\(^2\) to 150 J/cm\(^2\), resulted in a decrease of surface PpIX
fluorescence from $1.20 \times 10^{-4}$ to $3.00 \times 10^{-6}$ of the maximum value – $P_{F(t=0)} = 6.80 \times 10^{-5}$ W – recorded before treatment, respectively. Furthermore, doubling the light dose (i.e. increasing treatment times by a factor of two) could increase the effective treatment initially achieved at a depth of 2.70 mm (75 J/cm$^2$) down to 3.30 mm (150 J/cm$^2$). From the outset the primary concern was to investigate the issue of delivered treatment light dose to the tumour. This study has been shown to be a useful research tool, which has highlighted the possibility of optimising PDT treatment regimes for deep tumours by delivering a larger treatment light dose to the tumour [6].

Interestingly, MAL-PDT – currently the only approved regime for superficial NMSC in Europe – employs a treatment light dose of 37 J/cm$^2$ – as recommended by the manufacturer – when using an irradiance of approximately 80 mW/cm$^2$ from the Aktilite as previously shown in Chapter 6, Figure 6.1 (d) and Figure 6.2. However, due to concerns relating to clearance rates, this light dose was increased to 75 J/cm$^2$ at the Photobiology Unit, Ninewells Hospital and Medical School, Dundee. Also, at our centre, ALA-PDT employs a treatment light dose of 75 J/cm$^2$ using the same Aktilite light sources [7]. Clinically, the treatment light dose continues to be the main measurable quantity. However, the treatment light dose is not prescriptive as there is still no widely used and/or accepted standard [8].

The utility of the MCRT model was again illustrated in Chapter 6 when PDT light sources were modelled in order to gain an in-depth understanding of how light from these sources was distributed within a tumour during a simulated treatment. This study demonstrated that the narrow spectral emission of the Aktilite is advantageous for PDT in terms of delivering an effective PDD at depth in the tumour. Furthermore, the choice of wavelength for PDT was also assessed through a series of MCRT simulations. As expected, 630 nm offered superior tumour penetration and consequently greater PDD administered to the tumour at depth over 405 nm. Modelling the transport of light in tissue with the MCRT method offers practical insights into PDT of NMSC, which can constructively assist in optimising treatment parameters.
7.2 Future Work

Research in PDT has certainly not been exhausted. Knowledge of other treatment parameters within the clinical setting, such as tissue oxygenation and optimal photosensitiser pro-drug concentrations are not yet precisely known and there is no conclusive evidence to suggest otherwise. The time delay between photosensitiser pro-drug delivery and the onset of irradiation also requires further investigation.

The success of PD at a research level has been highlighted throughout this thesis. The practical implications of this work bode well for routine clinical acceptance, suggesting that standard treatment protocols could be improved by future work on PpIX fluorescence. There is evidence to suggest that MAL and ALA have limited penetration depths through tumour tissue [9]. Interestingly, increasing the pro-drug application time, may enhance its uptake and penetration into cancerous cells [10]. ALA penetration depths of between 2 and 5 mm have been reported in the literature [9,11,12]. Further studies of this are warranted and present other research opportunities where the spatial variations in the distribution of PpIX within a tumour could be assessed by fluorescence measurements and histological samples.

Current photosensitiser pro-drug application times are associated with a degree of inconsistency and there is uncertainty as to whether existing time delays before light irradiation offer maximal treatment outcomes. An interesting study would be to compare the time course accumulation – ranging over several hours – of ALA- and MAL-induced PpIX fluorescence within tumours. This may assist in identifying other time points were the onset of irradiation may be even more beneficial. Previously published studies pertaining to normal skin [13–15] should prompt future studies investigating the characteristics of ALA- and MAL-induced PpIX fluorescence in tumour tissue. More studies are required to determine whether ALA or MAL is superior for common use in PDT. However, the results presented in this thesis offer no clear significant difference between both pro-drugs.

PDT–induced pain can be significant for some patients and therefore may limit successful delivery of this treatment. There are various precautions to safeguard against severe pain. These include distracting the patients by talking to them, air cooling and subcutaneous anaesthesia [7]. However, pain still remains a major determinant preventing PDT from becoming an even popular treatment among patients affected by NMSC. Promising new developments are underway, with PDT
being performed using lower irradiances. Low-irradiance ambulatory PDT is an exciting new approach to delivering PDT [16]. In this instance, a light dose of 75 J/cm\(^2\) is delivered at an irradiance of 7 mW/cm\(^2\) over 3 hours [17]. Low irradiance PDT in comparison to higher irradiances was shown to enhance photobleaching efficiency and the PDT effect [18]. Furthermore, Wiegell et al. [19], suggested that low irradiance daylight PDT is less painful with similar efficacy than conventional PDT for AK. Attili et al. [20], have presented promising preliminary results highlighting a median pain score of 2 on the visual analogue scale, which is a substantial improvement on a median pain score of 6 experienced with conventional PDT. To date, low-irradiance ambulatory PDT has offered encouraging results. Therefore, it is expected that this PDT delivery technique will challenge conventional PDT in the near future. Although, not applicable for all lesions, particularly lesions > 2 cm, low-irradiance ambulatory PDT enables patients to leave the hospital after application of the pro-drug and light source. This reduces the patients’ total time spent in clinics. Plans are underway and it is expected that a comparative study between low and high irradiance PDT will follow in the near future. Potential primary and secondary end-points could be defined as PpIX fluorescence, pain and long-term treatment outcomes. Also, fractionation of light delivery may be beneficial to patients as there is evidence to suggest that light fractionated ALA-PDT offers high complete response rates for the treatment of NMSC [21]. Moreover, studies are necessary in order to gain more of an insight into oxygen measurements recorded in vivo during PDT [22]. On-going efforts are being made to measure \(^1\)O\(_2\) directly under clinical conditions. Undoubtedly, this would have a significant positive impact on PDT dosimetry.

An extension to the work presented in Chapter 3 and Chapter 5 could be to use the OBS to investigate PpIX fluorescence photobleaching at even earlier times and with more data points. This would be interesting as it may provide further information into how the photobleaching curve decays. Tyrell et al. [3], have demonstrated the potential of employing PpIX fluorescence photobleaching as a predictive tool for determining clinical outcomes.

Further studies should endeavour to mathematically model different tumour shapes and sizes, which would serve as an indicator of how light penetrates through tumours presenting with complex and possibly more realistic dimensions. When
modelling the distribution of PpIX within tumours, it is generally accepted to assume a homogeneous distribution. Further investigations would involve incorporating a depth-dependent PpIX distribution. This may represent an even more realistic clinical situation.

Modelling low-irradiance PDT is another interesting research option. However, there is a need at this time for data on clinically determined $^1$O$_2$ production.

There is an apparent lack of well-established data pertaining to the optical properties of NMSC. MCRT modelling could be used to determine optical properties data sets over a wide range of wavelengths, which should inform PDT treatment protocols. This would facilitate tailoring protocols for individual requirements. This is important as it is well known that optical properties have an effect on the distribution of light within a tumour. It is recommended that optical properties should be taken into account when devising study protocols. However, this is not always practical from a clinical point of view.

Other avenues of research include PD and PDT of brain tumours, disorders of the urinary tract and lung cancers. These are currently being performed at Ninewells hospital and studies into optimising treatment for these oncologic indications are ongoing. Optical fibres are employed in order to diagnose and treat these malignant tumours. PD enables guided biopsy along with controlled or complete resection and assists in normal tissue sparing [23]. Accurate determination and knowledge of light distributions pertaining to optical fibres are necessary before being used in clinical PDT. From an experimental standpoint, measurements of light distributions are currently being carried out using optical fibres. This should assist in defining the light dose administered during PDT regimes and be transferable into clinical settings.

Finally, Rajaram et al. [24], have shown that the combination of optical diagnostic techniques, such as LIFS and DRS can classify NMSC with increasing sensitivity and specificity. It has been demonstrated that applying ALA affects the Raman spectra of bladder tissue [25]. Therefore, the potential of combining LIFS and RS in order to reduce false positives may assist in improving in vivo diagnosis further.
7.3 Conclusion

While the current status of PDT for NMSC is encouraging, there is clearly promise for the future. The work presented in this thesis has demonstrated the potential clinical impact of PD and MCRT modelling on PDT, particularly for NMSC. Although PDT is widely and effectively used, and the treatment of choice for selected lesions, it has not yet reached its full potential. This thesis with its concomitant publications has endeavoured to compliment and further understanding of PDT in the clinical setting. Critical aspects of PDT include standardising treatment protocols together with more accurate dosimetry. Increasing knowledge and understanding of treatment parameters through clinical studies and mathematical modelling, together with the management or prevention of pain will hopefully result in improved clinical PDT treatment protocols for NMSC and other oncologic indications.
7.4 References


Chapter 7 – Concluding Remarks


