Biological and physical strategies to improve the therapeutic index of Photodynamic therapy

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Medical Biophysics
University of Toronto

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Abstract

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Photodynamic therapy (PDT) derives its tumour selectivity from preferential photosensitizer accumulation and short light penetration in tissue. However, additional strategies are needed to improve the therapeutic index of PDT in oncological applications where light is delivered interstitially to large volumes (e.g. prostate), or when adjacent normal tissue is extremely sensitive (e.g. brain). Much research to improve PDT’s selectivity is directed towards developing targeted photosensitizers. Here, I present two alternative strategies to improve PDT’s selectivity, without compromising its efficacy. For interstitial delivery, I investigated whether customizable cylindrical diffusers can be used to deliver light doses that conform better to target geometries, specifically the prostate. Additionally, I examined whether the neuroprotectant erythropoietin, used as an adjuvant to PDT for brain tumours, can reduce the sensitivity of normal tissue, thereby improving treatment selectivity.

To determine if tailored diffusers constitute an improvement over conventional ones, I introduce a novel optimization algorithm for treatment planning. I also analyze the sensitivity of the resulting plans to changes in the optical properties and diffuser placement. These results are contextualized by a mathematical formalism to characterize the light dose distributions arising from tailored diffusers. In parallel, I investigate the neu-
I show that the most important parameter determining prostate coverage is the number of diffusers employed. Moreover, while tailored diffusers do offer an improvement over conventional ones, the improvement is likely masked by perturbations introduced by the uncertainties of light delivery. Although these results largely discard the use of tailored diffusers in prostate PDT, significant insight has been gained into PDT treatment planning, and tailored diffusers may still be advantageous in more complicated geometries. Additionally, I show that erythropoietin does not improve survival of PDT-treated neurons in PDT, nor reduces the volume of necrosis in vivo, for the ranges of conditions and doses studied. To our knowledge, this is the first time this strategy has been tested in brain PDT and deserves to be investigated further, by using later time-points, functional outcomes, and other neuroprotectants.
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I would like to dedicate this thesis to my wife Mellone Marchong and thank her not only for these few years that she has supported and encouraged me, both emotionally and scholarly, but also for the many more years to come. It is a great treasure to have someone so close that understands the problems and helps point out the solutions.

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Nomenclature

Acronyms

ALA 5-aminolevulinic acid

AMD Age-related Macular Degeneration

BPD-MA Benzoporphyrin derivative monoacid A-ring (Verteporfin)

CT Camptothecine

DVH Cummulative Dose Volume Histogram

EPO Erythropoietin

EPOR Erythropoietin Receptor

hrEPO human recombinant Erythropoietin

IGF-I Insuline-like Growth Factor I

IU International Units

JAK2 Janus tyrosine kinase 2

L(H)DL Low(High) densitiy lipoproteins

NPe$_6$ Mono-aspartyl chlorin e$_6$

m-THPC Meta-tetrahydroxyphenyl chlorin (Foscan)
<table>
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<tr>
<td>NGB</td>
<td>Naphthol Green B</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NTCP</td>
<td>Normal Tissue Complication Probability</td>
</tr>
<tr>
<td>PDT</td>
<td>Photodynamic Therapy</td>
</tr>
<tr>
<td>PpIX</td>
<td>Protoporphyrin IX</td>
</tr>
<tr>
<td>PS</td>
<td>Photosensitizer</td>
</tr>
<tr>
<td>R(L)HS</td>
<td>Right(Left) hand side</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RTF</td>
<td>Radiation transfer equation</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
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<tr>
<td>SP</td>
<td>Staurosporine</td>
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<tr>
<td>SVD</td>
<td>Singular Value Decomposition</td>
</tr>
<tr>
<td>TCP</td>
<td>Tumour Control Probability</td>
</tr>
<tr>
<td>TI</td>
<td>Therapeutic index</td>
</tr>
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**Symbols**

- $\kappa$: Condition number
- $f$: Cost function
- $d^L$: Minimum effective dose [Wcm$^{-2}$]
\( d^U \) Maximum permissible dose [W cm\(^{-2}\)]

\( E(r, \Omega) \) Irradiance [W cm\(^{-2}\) sr\(^{-1}\)]

\( F(r) \) Flux (or current) [W cm\(^{-2}\)]

\( \Phi(r) \) Fluence rate [W cm\(^{-2}\)]

\( G(r) \) Point source function [cm\(^{-2}\)]

\( g \) Average cosine of the scattering phase function

\( L(r, \Omega) \) Radiance [W sr\(^{-1}\) cm\(^{-2}\)]

\( \mu_a \) Absorption coefficient [cm\(^{-1}\)]

\( \mu_{\text{eff}} \) Effective attenuation coefficient [cm\(^{-1}\)]; \((3\mu_a\mu_{\text{tr}})^{1/2}\)

\( \mu_s \) Scattering coefficient [cm\(^{-1}\)]

\( \mu_t \) Total attenuation coefficient [cm\(^{-1}\)]; \(\mu_a + \mu_s\)

\( \mu_{\text{tr}} \) Transport coefficient [cm\(^{-1}\)]; \(\mu_a + \mu_s(1 - g)\)

\( p(\Omega, \Omega') \) Scattering phase function

\( r \) Vector pointing to observation point

\( S(z') \) Diffuser emission profile [W cm\(^{-1}\)]

\( S(r, \Omega) \) Source of irradiance [W cm\(^{-3}\) sr\(^{-1}\)] (see footnote in page 17)

\( \Omega \) Unit vector in direction of light propagation
Chapter 1

Introduction

At the heart of any therapeutic intervention is the therapeutic index (TI). Figure 1.1 shows hypothetical curves for the tumour control probability (TCP) and the normal tissue complication probability (NTCP) as a function of dose. The TI measures the ratio of the doses required to achieve, with equal probability, tumour control and tissue complications (e.g. $Dose_{NTCP=0.5}/Dose_{TCP=0.5}$ as indicated). The TI thus measures the horizontal separation of these two curves. The treatment’s selectivity is related to the ratio TCP/NTCP and varies with dose, and in turn, with efficacy. Therefore, the success of any therapy is not solely dictated by the selectivity. In principle, very high selectivities can be achieved for low doses but at the cost of treatment efficacy. The ultimate goal of any research geared towards improving an existing treatment modality is to spread these curves further apart, that is, to improve the therapeutic index.

Photodynamic therapy (PDT) is a treatment modality for cancer and other conditions. It involves administering photosensitizing drugs, called photosensitizers (PS), and photochemically exciting them by illuminating with visible or near infrared light at wavelengths determined by the PS absorption spectrum. In the abundance of oxygen, the energy of the excited PS is transferred to oxygen molecules, leading to the generation of reactive oxygen species (ROS) [Henderson & Dougherty 1992], which are ultimately responsible for the cytotoxicity of PDT. In summary, the PDT dose depends on the PS
Figure 1.1 – Depiction of the concepts involved in the definition of the therapeutic index (TI). Hypothetical TCP (solid) and NTCP (dashed) curves are shown. The TI ($D_{NTCP=0.5}/D_{TCP=0.5}$) measures the separation between these two curves. Selectivity is given by the ratio TCP/NTCP, at a given dose. Notice that, typically, low doses display good selectivity but are not very effective; in turn, the selectivity decreases at more effective doses.

PDT’s selectivity is mostly due to the preferential uptake of the PS by the tumour [Jori 1996]. This selectivity has been exploited for successfully treating a variety of skin conditions [Babilas et al. 2006], cancers of the head and neck [Biel 2006], and premalignant and early esophageal cancer [Dacosta et al. 2006, Tokar et al. 2007], among others. Although not necessarily true, a comparison between different photosensitizers seems to obey the relationship between efficacy and selectivity depicted in fig. 1.1, PS that display exquisite selectivity typically have poor efficacy (e.g. ALA [Madsen et al. 2006]), and those that are highly effective tend to be unselective (e.g. m-THPC [Radu...].
PS with high selectivity are still very useful for fluorescence guided detection and resection [Stepp et al. 2007, Smolka et al. 2006, Bogaards et al. 2005]. Naturally, the goal of photosensitizer development is to improve selectivity and efficacy, that is, the TI.

A second level of selectivity is available from the reduced penetration of light in tissue. This allows the selective illumination of small volumes of tissue while sparing potentially healthy tissue. This additional selectivity has been exploited for treating lesions of epithelial origin [Etienne et al. 2004, Grosjean et al. 1998]. Short light penetration, however, presents a dilemma in terms of efficacy. While for superficial lesions one can ensure that the complete target volume receives a sufficient light dose, for larger target volumes, and particularly for deep-seated tumours requiring interstitial light delivery, short light penetration hinders adequate coverage of the complete target.

With these hurdles in mind, there are at least two scenarios where one would like to improve the TI while maintaining the efficacy of PDT. On the one hand, some PS, such as WST09 and BPD-MA are very effective but not very selective. One would like to recover the TI for such potent PS by improving the selectivity of the light delivery. On the other hand, PS such as Photofrin and ALA are tumour selective; however, the surrounding normal tissue may be far more sensitive to PDT than the tumour itself [Lilge & Wilson 1998], and therefore the overall PDT selectivity is drastically reduced. Such is the case of brain tissue; therefore, a possibility to recover the TI is to decrease the sensitivity of normal tissue.

The great majority of the research towards improving the efficacy and TI of PDT is directed towards synthesizing novel PS that are selective and potent [Solban et al. 2006]. Clearly, other approaches to improve the TI, such as the two options outlined above, can be of great benefit, as they increase the arsenal of therapeutic strategies against cancer, complementing the ongoing development of new PS. These two strategies will be the subject of this dissertation.
Chapter 1. Introduction

1.1 Mechanisms of action of PDT in vivo

PDT destroys tumours by three main mechanisms: vascular shutdown, direct cell kill, and processes involving the immune system. Before we look at these mechanisms in vivo, let us first briefly review the photochemical reactions that lead to the initiation of these mechanisms.

1.1.1 Photochemistry of PDT

Photosensitizes are designed such that absorption of a photon by the PS in its singlet ground state ($^1P$) leads to a long lived excited triplet state ($^3P^*$). In the abundance of oxygen, the excitation of $^3P^*$ can be transferred to oxygen ($^3O_2$), leading to the formation of singlet oxygen ($^1O_2$), an extremely reactive ROS, and the PS returning to $^1P$. In hypoxic or anoxic conditions, because of the low abundance of oxygen, the excited PS ($^3P^*$) more likely oxidizes biological substrates by electron transfer. The resulting reduced PS ($P^-$) can still react with oxygen (in hypoxic conditions) to produce superoxide anions ($O_2^-$), which in turn can produce the radical hydroxyl ($OH^-$). Alternatively, the excited PS ($^3P^*$) can also react with superoxide radicals ($O_2^-$) to produce superoxide anions ($O_2^-$), and thereby, the hydroxyl radical ($OH^-$). Foote [1991] originally defined the latter reactions, which depend on the substrate concentration, as type I reactions, while the former ones leading to the generation of singlet oxygen were called type II. Together, these oxidative molecules are referred to as ROS. Further details can be found in reviews by Henderson & Dougherty [1992], Ochsner [1997], Macdonald & Dougherty [2001].

In general, type II reactions are preferred for the high reactivity of singlet oxygen and because the PS molecule is, in principle, photochemically recycled by returning to its ground state, rather than being lost as one of the reaction products. The recycling of the PS reduces photobleaching (loss of the PS due to illumination) [Georgakoudi & Foster 1998], potentially improving the efficacy and facilitating the dosimetry. These properties
of type II reactions are also responsible for the improved tumour response to PDT under normoxic conditions [Pogue & Hasan 1997], and explain, in part, why hypoxic or poorly vascularized tumours are less responsive to PDT [White et al. 1988, Henderson & Fingar 1987, Chen et al. 2005b]. Overall, the relative contributions of type I and II reactions to the photodynamic process depend on the oxygen concentration, and consequently on the rate of delivery of PDT (due to oxygen consumption by PDT) [Henderson et al. 2006]. However, type II mechanisms appear to be more common [Henderson & Dougherty 1992].

The main biological targets of singlet oxygen in PDT are proteins and membranes (lipids). The life time of singlet oxygen in vivo is between 30 and 180 ns, which corresponds to a diffusion range less than 50 nm [Niedre et al. 2002, Moan 1990]. Thus, the intracellular localization of the PS determines the cellular structures that are attacked by singlet oxygen [Peng et al. 1996]. Most proteins are severely damaged by the products generated during PDT, ultimately leading to loss of their biological activity [Ochsner 1997]. Cellular membranes are also a major target for photosensitized oxidation. Damage to several cellular structures has been observed, including the plasma and lysosomal membranes [Shulok et al. 1986], mitochondria [Morgan & Oseroff 2001], and endoplasmic reticulum [Kessel & Reiners 2007].

Singlet oxygen can also damage DNA. However, since PS localization determines the site of damage, singlet oxygen does not diffuse far from the site of production, and the great majority of PS do not reach the nucleus, PDT-mediated DNA damage is extremely rare, explaining the lack of mutagenicity observed following PDT [Ben-Hur et al. 1987]. The absence of DNA damage also accounts for the relatively low efficacy of PDT compared to DNA-damaging therapeutic agents such as chemotherapy and ionizing radiation. In fact, targeting PS to the nucleus can enormously increase its cytotoxicity [Akhlynina et al. 1997].

\footnote{It is worth noting that recent work by Hatz et al. [2007] has questioned the lifetime of singlet oxygen placing it in the 3 µs range. This new results have generated plenty of controversy that has not been successfully clarified as of yet.}
1.1.2 Vascular shutdown

The importance of vascular damage for tumour destruction is well recognized. Studies by Henderson et al. [1985] revealed that if EMT-6 or RIF tumours grown in mice where excised immediately following PDT (with dihematoporphyrin ether), clonogenicity was nearly unaffected. However, excising tumours at later timepoints resulted in decreased survival that mirrored the survival of cells directly exposed to anoxia for equal amounts of time. More recently, Fingar et al. [1999] showed that tumour regression following BPD-MA mediated PDT was greatest when the drug light interval was chosen to maximize vascular shutdown.

Photosensitization within the vasculature leads to increased vascular permeability, vasoconstriction and thrombus formation [Fingar et al. 1997, 1999, Dolmans et al. 2002], which ultimately results in ischemia and tissue necrosis. PDT is thought to expose the vascular basal membrane by damaging the cytoskeleton of the endothelial cells that lining the vessel [Chaudhuri et al. 1987, Sporn & Foster 1992]. Basal membrane exposure enables platelet adhesion and activation. As a result, multiple eicosanoids are released to mediate vasoconstriction and enhance thrombus formation [Fingar 1996]. Of these, thromboxane is thought to be a key modulator, given the ability of indomethacine (an inhibitor of the synthesis of prostaglandins) to decrease vascular damage following PDT, and that prostacyclin (another prostaglandin) serum levels remain unaffected during PDT [Reed et al. 1989, Fingar et al. 1990]. In addition to thromboxane, damage to endothelial cells promotes the release of leukotrienes, which cause increased vessel permeability and leukocyte recruitment [Fingar et al. 1991]. The ensuing oedema increases interstitial pressure, further constricting blood vessels. The combination of vessel constriction, thrombus formation, and increased interstitial pressure leads to tissue ischemia followed by necrosis.
1.1.3 Direct cell kill

Direct cell kill refers to the activation of the PS directly within cells. Adams et al. [1999] have beautifully demonstrated that this type of cell kill is an important contributor to PDT mediated tumour destruction \textit{in vivo}. The authors used RIF cells that had been made resistant to Photofrin-PDT by multiple rounds of PDT \textit{in vitro} [Singh et al. 1991], and demonstrated that tumours formed by these cells were more resistant to PDT \textit{in vivo} than their parental line, despite that vascular damage between them was similar.

Direct cell kill can occur through necrosis or apoptosis. Which fate the cell undergoes mainly depends on cell type, PS, incubation protocol, and photodynamic dose [Noodt et al. 1996, Wyld et al. 2001, Noodt et al. 1999, Bourr et al. 2002]. Given the wide spectrum of molecular targets damaged by PDT, it is not surprising that many pathways have been implicated in the cellular response to PDT. Necrosis usually occurs subsequent to plasma membrane disruption, mitochondrial destruction leading to energy depletion, disruption of lysosomes leading to autophagy, or simply generalized cellular damage.

Apoptosis has been also identified as a form of cell death following PDT. Multiple PS preferentially accumulate in the mitochondria (e.g. Photofrin and ALA induced Protoporphyrin IX – PpIX). Given the central role of this organelle in apoptosis control, it is not surprising that PDT can indeed lead to this mode of cell death [Kessel & Luo 1999]. Damage to multiple organelles and the activation of several biochemical pathways leading to apoptosis have also been implicated following PDT. These processes have been the subject of multiple reviews and will not be addressed here (see for example [Moor 2000, Oleinick et al. 2002, Plaetzer et al. 2005, Buytaert et al. 2007]). Despite the prevalence of reports providing explanations for the mechanisms of action of PDT that lead to apoptosis, it is important to note that the great majority of these studies have been performed \textit{in vitro}. The relevance of these pathways during PDT \textit{in vivo} is far less understood.
It is difficult to study the contribution of apoptosis to the overall tumour destruction \textit{in vivo} due to the rapid clearance of apoptotic cells by macrophages. Despite this, apoptosis following PDT has been observed in multiple experimental animal models (e.g. [Zaidi \textit{et al.} 1993, Whitacre \textit{et al.} 2000, Lilge \textit{et al.} 2000] among many others). Moreover, since the molecular changes during apoptosis are relatively well understood, and markers for these events are available, recent studies have attempted to use apoptosis as a measure of tumour response to treatment. Cauchon \textit{et al.} [2007] have recently used PET imaging with a radionuclide targeted to phosphatidyl serine to visualize the occurrence of apoptosis following PDT of tumour bearing mice. Notably, Stefflova \textit{et al.} [2006] have reported the use of a multifunctional molecule that combines a PS with an apoptosis fluorescence reporter based on caspase-3 cleavage. Whether such strategies will be good predictors of treatment outcome remains unknown. The importance of vascular mechanisms of tumour destruction in PDT and the poor correlation between apoptosis and tumour response seen in other treatment modalities [Tannock & Lee 2001] question the validity of these approaches.

1.1.4 Immune-mediated effects

PDT can activate or suppress the immune system depending on the type of PS and PDT dose [Korbelik 1996, 2006]. Macrophages seem to play a central role in linking PDT and the immune system. They accumulate large amounts of PS, often more than tumour cells [Korbelik \textit{et al.} 1991]. Furthermore, upon photostimulation and depending on the PDT dose, they may regulate the release of pro- or anti-inflammatory cytokines [Lynch \textit{et al.} 1989, Korbelik & Kros 1994].

In addition to the inflammatory processes that can lead to further cell kill by the action of neutrophils, mast cells, monocytes and macrophages, PDT is able to induce long-term specific immunity, suggesting a role in improving antigen presentation or T-cell activation [Korbelik & Cecic 1999, Korbelik & Dougherty 1999, Hendrzak-Henion
et al. 1999]. Moreover, cells inactivated by PDT have been shown to be highly effective when used as anticancer vaccines in comparison with cells inactivated by UV, γ-radiation, or lysis by freeze-thaw cycles [Gollnick et al. 2002]. Castano et al. [2006] have recently reviewed the involvement of the immune-system in the modulation of the tumour response to PDT.

1.2 Strategies to improve the selectivity of PDT

The strategies to improve the selectivity of PDT can be divided in three categories: PS selectivity, light delivery, and modification of the tissue response. This section will describe the basis for the selectivity of these approaches and provide some examples.

1.2.1 Photosensitizer selectivity

Treatments with very high selectivity towards malignant tissue are well suited for cancers with poorly defined margins (e.g. gliomas), multi-focal characteristics (e.g. some skin cancers), or where the anatomical complexities make appropriate light dosimetry too cumbersome (e.g. peritoneal cavity). In such cases light dosimetry is insufficient to provided the required selectivity.

PS selectivity is measured by the uptake ratio between tumour and surrounding tissue. For most PS with passive tumour selectivity (see below), the uptake ratio is between 1 and 10 (typically between 1-3) depending on tumour-PS combination and time after administration [Jori 1996]. Much higher uptake ratios can be found in the brain, particularly with ALA [Lilge & Wilson 1998], mainly due to the inability of ALA to cross the intact blood brain barrier [Ennis et al. 2003]. In fact, many PS display better selectivities in the brain than in other locations for this same reason.

There are at least two main approaches to improve the selectivity of a photosensitizer: passive and targeted strategies. Traditionally, selectivity has relied on designing PS that
have a passive preferential tumour uptake. An active strategy to improve PS selectivity employs antibodies, peptides and other molecular constructs to target the PS to the tumour.

The attenuation of light in tissue leads to very steep gradients in the light dose (around 100 fold reduction per cm), which has important consequences in terms of PDT selectivity. For example, if tumour pockets are behind 1 cm of normal tissue along the light path, an uptake ratio of 10 still leads to 10 times higher PDT dose delivered to the normal tissue (product of uptake and light dose ratios). Other strategies beyond passive uptake are thus needed to improve the selectivity of PDT.

**Passive selectivity**

The mechanisms behind passive PS uptake are still not fully understood, and several factors may play important roles. On the tumour side, leaky vasculature, compromised lymphatic drainage, the abundance of low density lipoprotein (LDL) receptors [Allison et al. 1990, 1991], and the reduced pH of tumours [Böhmer & Morstyn 1985, Moan et al. 1980] all contribute to higher PS accumulation and longer retention times. On the PS side, better selectivity is observed for compounds with higher lipophilicity (strong binding to LDL and HDL in plasma), and amphiphilicity (likely due to easier accumulation in membranes). However, exceptions to these rules are not uncommon.

Reflecting the dynamic character of the uptake process, the time between drug administration and light delivery is critical. Typically, the PS is cleared from tissues and plasma by 24 h. Thereafter, due to the slower clearance rate in tumours, the uptake ratio increases with the drug light interval [Ris et al. 1993]. When a favourable uptake ratio has been achieved light can be delivered, often taking several days between drug administration and illumination (e.g. 2 days for Photofrin and 4 for m-THPC).

The PS distribution between the vascular and cellular compartments of a tumour also depends on time. Chen et al. [2005a] clearly demonstrated this in a rat prostate tumour
model, where the tumour distribution of BPD-MA following PS injection changed from predominantly vascular (15 min), to diffuse throughout the tumour parenchyma (3 h). They also showed that the efficacy of PDT in these regimes was different, with early illumination resulting in better tumour control as measured by an at least four-fold reduction in tumour burden.

A common strategy to improve the biodistribution of PS is their encapsulation in various delivery vehicles such as liposomes, oil-dispersions and polymeric particles [Konan et al. 2002, 2003, Chen et al. 2006a]. The encapsulation not only has been found to improve the biodistribution, but is often required with more hydrophobic PS that are not water soluble. For example, the liposomal formulation of BPD-MA has higher uptake in tumour than the non-liposomal one [Richter et al. 1993], likely due to preferential accumulation in the neovasculature that expresses high numbers of LDL receptors. This approach was instrumental in the therapeutic success of BPD-MA (Visudyne) for the treatment of age-related macular degeneration (AMD) [Renno & Miller 2001].

Drug concentration is also an important modulator of selectivity. Recently, Oseroff et al. [2006] have reported the results of a large dose ranging study for Photofrin-PDT used for treating basal cell carcinoma. The authors found that the largest concentrations of Photofrin (1 mg/kg) presented the best tumour response rates, however, at a slight cost in selectivity. This, of course, serves to remind us of the intrinsic relationship between efficacy and selectivity illustrated in fig. 1.1.

Targeting constructs

The growing body of knowledge of the molecular biology of cancer and the identification of tumour specific markers has enabled the development of tumour targeted PS. PS have been conjugated to a variety of monoclonal antibodies raised against tumour markers, most notably the epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), vascular endothelial growth factor (VEGF), carcino-embryonic
antigen (CEA), and several cluster of differentiation molecules (CD) [van Dongen et al. 2004, Solban et al. 2006]. In addition to antibodies, peptides targeted to specific receptors (e.g. EGF, VEGF) have also been conjugated to PS [Sharman et al. 2004, Schneider et al. 2006, Taquet et al. 2007].

Another recent approach termed “killer beacons” combines a PS and a quencher joined by DNA or peptide linkers. The linkers are disease specific sequences that, for example, can be cleaved by cancer specific proteases such as metalloproteinases [Zheng et al. 2007] or anneal to particular nucleotide sequences. Under normal conditions, the linker is folded such that the PS and quencher are close together, thereby inhibiting the production of singlet oxygen. Once in the tumour, the PS and quencher are driven apart by the linker target, enabling photoactivation, production of singlet oxygen, and tumour cell kill [Stefflova et al. 2007].

While many of these targeting constructs display excellent specificity in vitro, their selectivity and efficacy in vivo is still under investigation. Furthermore, many of them are likely to suffer from insufficient biodistribution due to the larger sizes of the molecules.

### 1.2.2 Light

When the target area is well demarcated, significant selectivity can be gained by appropriate light dosimetry. For superficial lesions, the treatment depth can be reduced by illuminating with light of shorter wavelengths. This strategy has been investigated for treating the oesophagus and bronchi with Photofrin [Grosjean et al. 1998]. The authors reported that illuminating with 514 nm light had the same probability of success as 630 nm light, albeit with a lower risk of perforation. Etienne et al. [2004] have used 514 nm irradiation with m-THPC in human subjects for early stage neoplastic lesions in Barrett’s oesophagus with excellent efficacy and moderate side effects. In a study involving sheep oesophagus using m-THPC (a more potent PS), Radu et al. [2003] noted that 413 nm light caused significantly less damage than 514 nm light, particularly at high fluences of
175 to 250 J/cm², and concluded that green light led to severe complications that would likely occur in humans as well. Other investigators using ALA-PDT in rat oesophagus found that low fluence rates and 633 nm, in comparison with 532 nm light, and higher fluence rates, in fact led to more selective epithelial damage [van den Boogert et al. 1999]. This latter study highlights that additional selectivity can be gained not only from optimizing the illumination wavelength but also the total fluence and fluence rate. These studies demonstrate the possibility to confine the PDT treatment in superficial or intraluminal geometries. The sheep studies, however, do emphasize the importance of exerting great care during light dosimetry, since the light confinement may not be sufficient to avert risk.

Using shorter wavelengths is counterproductive for interstitial sites that require multiple light sources to ensure adequate coverage. In these cases, optimal placement of sources can be used to selectively irradiate the target geometry. A few studies have used computer assisted technology to guide the source placement. Recently, Beck et al. [2007] have used computer visualization to guide the placement of cylindrical diffusers for illumination of brain tumours treated with ALA-PDT in ten patients. The authors found this delivery approach very satisfactory in terms of low morbidity, with no oedema nor decrease of neurological function, also reflecting the few side effects of ALA-PDT for brain tumours.

Other investigators have used computer based treatment planning for PDT of the prostate [Altschuler et al. 2005, Johansson et al. 2007b, Weersink et al. 2005]. Notably, Altschuler et al. [2005] and Johansson et al. [2007b] have developed computer optimization strategies to determine the best possible positions and output powers for the sources. Given the complexity of PDT dosimetry, computer-based treatment planning and on-line dose monitoring will likely become an integral part of future PDT delivery systems. This kind of selectivity is the subject of Chapter 2.
A final word regarding light dose fractionation is in order. In radiation therapy, dose fractionation is one of the main mechanisms to improve the TI. In PDT, however, it is used to restore efficacy lost by treatment-induced oxygen depletion and, for ALA, to allow for photosensitizer resynthesis [Gibson et al. 1990, Curnow et al. 2000, van Geel et al. 1996, van den Boogert et al. 2001]. The fractionation intervals in PDT range from seconds to a few hours. Interestingly, Bisland et al. [2004] have proposed a metronomic (i.e. continuous very low dose rate) PDT regime to improve the selectivity of PDT for brain tumours. This strategy is based on the observations that, at low doses, PDT causes very selective tumour cell death via apoptosis in a rabbit brain tumour model via implantation of VX2 sarcoma cells in the brain [Lilge et al. 2000].

1.2.3 Biological response

In addition to manipulating the selectivities of the PS and light delivery, other strategies aim at modifying the tissue response to PDT. Many of the latter strategies are more directed towards increasing the efficacy of PDT, although in some cases, the selectivity has also been found to improve.

Since PDT is less effective in hypoxic tumours, increasing tumour oxygenation is expected to improve selectivity. Increasing tumour oxygenation increases the efficacy of PDT, assuming that normal tissue oxygenation, and thus the PDT dose to normal tissue, remains unchanged. An approach to improve tumour oxygenation has been using hyperbaric oxygen or carbogen breathing [Huang et al. 2003, Chen et al. 2002a, Maier et al. 2000]. A majority of studies have found that indeed the efficacy of PDT is improved, but whether the selectivity is also increased is less clear.

PDT mediated cytotoxicity is highly dependent on ROS. Not surprisingly many strategies have tried to improve the efficacy of PDT by reducing the cellular response to oxidative stress. Jiang et al. [1998] have used buthionine sulfoximine (BSO), an inhibitor of glutathione synthesis, precisely to reduce the response capacity to oxidative
stress. The authors measured the depth of necrosis in normal brain and two brain tumour models and found that BSO in combination with Photofrin not only potentiated the effects of PDT, but also resulted in higher tumour selectivity. They suggested that the added selectivity may have come from the differential entry rates of BSO into tumour and normal brain. This interesting hypothesis opens the door to further improve PDT’s selectivity and efficacy by using other enhancers that, similarly, are unable to cross the blood brain barrier.

Another exciting strategy is to directly activate the immune system to target tumour cells. Korbelik et al. [1997] administered vitamin D$_3$-binding protein-derived macrophage-activating factor (DBPMAF), an activator of macrophages, in combination with Photofrin PDT of a mouse tumour model of squamous cell carcinoma and found that the adjuvant boosted cure rates from 25 %, in the absence of DBPMAF, to 100 %. In the same spirit, multiple studies have combined PDT with a variety of bacterial-derived immunoadjuvants [Myers et al. 1989, Uehara et al. 2000, Krosl & Korbelik 1994], as well as pro-inflammatory cytokines [Bellnier 1991, Golab et al. 2000], to stimulate the immune system to attack the tumour. Furthermore, other investigators have pursued strategies to enhance the cellular arm of the anti-tumour immune response by either depleting T-regulatory cells that suppress the immune response using cyclophosphamide [Castano & Hamblin 2005], or by promoting tumour antigenicity by injecting dendritic cells [Jalili et al. 2004, Saji et al. 2006]. Overall, the results of these immuno-modulatory studies are very impressive in terms of improved tumour control. The enhanced selectivity of these strategies is promising for treating disseminated diseases [Castano et al. 2006].

A final strategy consists of selectively protecting normal cells from PDT using trophic factors. Paskowitz et al. [2007] have studied a variety of neurotrophic factors to minimize retinal toxicity following PDT for AMD and found that brain-derived neurotrophic factor (BDNF) resulted in the best protection. This kind of strategy can potentially be used in
other cancer sites such as the brain, where neurons are largely irreplaceable. This is the approach taken in Chapter 4.

1.3 Light propagation in tissue

This section introduces the theory of light transport in turbid media. In addition to explaining the origin of the radiation transport equation (RTE), I will present the standard method to solve the RTE by expanding the radiance in spherical harmonics, which leads to the diffusion equation for light transport in scattering media. A solution for a point source emitting in an infinite medium will be also presented. We will be concerned here only with light sources that emit constantly and therefore no reference to time appears in the equations. In other words, a steady state has been achieved by waiting long enough after turning on the light source.

The RTE is a heuristic approach to solve the problem of light propagating through matter. Rather than solving Maxwell’s equations, one ignores the effects of interference between waves scattered by the molecules in the medium, and considers the problem of photons being absorbed or colliding with scattering centres in the medium. The derivation of the diffusion equation shown below has been modified from Willem Star, in [Welch & van Gemert 1995, ch. 6]. In order to ensure consistency and avoid introducing notation that is only needed for more complicated geometries and boundary conditions, I have provided a solution of my own for the point source problem. The solution is based on the standard Green’s function approach.

One first defines the absorption and scattering probabilities for photons travelling an infinitesimal distance $ds$, as $\mu_a ds$ and $\mu_s ds$, respectively. With this in mind, one can define the probability of a photon being scattered or absorbed in a distance less than $s_1$ as

$$P(s < s_1) = 1 - \exp(-\mu_s s_1),$$
where $\mu_t = \mu_a + \mu_s$ is the total attenuation coefficient. These probabilities describe all the interactions of photons with the medium that we are interested in.

### 1.3.1 Radiation transport equation

The RTE accounts for what happens to a ray or light (or a packet of photons) as it interacts with the medium. The concept of a ray of light is embodied in the radiance $L(r, \Omega)$, which describes the amount of energy (number of photons) per unit of time, contained within an infinitesimal solid angle $d\omega$, centred around the direction $\Omega$, that is crossing an infinitesimal area at position $r$, perpendicular to $\Omega$. Its units are therefore [W cm$^{-2}$ sr$^{-1}$], where cm are used rather than meters because it better describes the order of magnitude of the processes taking place. Keeping track of what happens to a ray of light $L(r, \Omega)$ as it travels a distance $ds$ along direction $\Omega$ we say that

$$\frac{dL(r, \Omega)}{ds} = \Omega \cdot \nabla L(r, \Omega) = -\mu_a L(r, \Omega) - \mu_s L(r, \Omega) + \mu_s \int_{4\pi} p(\Omega, \Omega') L(r, \Omega) d\omega' + S(r, \Omega).$$

(1.1)

Term by term, this equation says that the change in the radiance along $ds$ in direction $\Omega$ is equal to the loss due to absorption and scattering along $ds$, plus the gain due to all other photons originally travelling along any direction $\Omega'$ that were scattered with probability $\mu_s p(\Omega, \Omega')$ in direction $\Omega$, plus a source term $S(r, \Omega)^2$. The term $p(\Omega, \Omega')$ is called the scattering phase function. It is normalized such that the total scattering probability equals one, that is: $\int_{4\pi} p(\Omega, \Omega') d\omega' = 1$. Furthermore, assuming that the medium is rotationally invariant, $p(\Omega, \Omega')$ depends functionally on $\Omega$ and $\Omega'$ only as $p(\Omega \cdot \Omega')$.

---

A note on notation. Traditionally, the notation for distance uses the letter $s$ and we adhere in this chapter to this convention. Here and in coming chapters, $S$, $s$, or $s_j$ are used to notate source terms. In this chapter $S(r, \Omega)$ is a source of radiance [W cm$^{-3}$ sr$^{-1}$], while in subsequent chapters $S(r')$ will signify a source power density [W cm$^{-3}$]. No confusion should arise as they are clear from the context.
We define two additional functions closely related to $L(r, \Omega)$. The fluence rate $\Phi(r)$ is obtained from $L(r, \Omega)$ by integrating over the complete solid angle:

$$\Phi(r) = \int_{4\pi} L(r, \Omega) d\omega .$$  \hfill (1.2)

It represents the flow of energy regardless of the direction. To obtain an average direction of the flow, one calculates the flux $F(r)$ as the weighted average of $\Omega$:

$$F(r) = \int_{4\pi} L(r, \Omega) \Omega d\omega .$$  \hfill (1.3)

These quantities are closely related to the zero- and first-order terms in the expansion of $L(r, \Omega)$ in spherical harmonics.

To obtain a functional form of the source term, it is useful to divide $L(r, \Omega)$ into two components, the primary and the scattered (or diffuse) radiance:

$$L(r, \Omega) = L_p(r, \Omega) + L_s(r, \Omega).$$  \hfill (1.4)

In the same way one can define $\Phi(r) = \Phi_p(r) + \Phi_s(r)$. Now, by definition, $L_p(r, \Omega)$ obeys eq. (1.1) with $p(\Omega, \Omega') = 0$, i.e.

$$\Omega \cdot \nabla L_p(r, \Omega) + \mu_t L_p(r, \Omega) = S(r, \Omega).$$  \hfill (1.5)

For an arbitrary source of radiance, the solution to this equation takes the form:

$$L_p(r, \Omega) = E_0(r) \exp(-\mu t) \delta(1 - \Omega \cdot \Omega_0)/(2\pi).$$  \hfill (1.6)

Here, $E_0(r)$ corresponds to the irradiance due to the source in the absence of tissue, for example $E_0(r) = 1/(4\pi r^2)$ for a point source emitting with unit power. The term $\exp(-\mu t)$ corresponds to the removal of photons from the primary path by absorption and scattering combined. Finally, the delta function accounts for the fact that the photons are only travelling along $\Omega_0$, which is the direction that joins the origin of the source and the point $r$. For a point source in an infinite medium the primary radiance takes the form:

$$L_p(r, \Omega) = \exp(-\mu t) \delta(1 - \Omega \cdot \Omega_r)/(2\pi).$$  \hfill (1.7)
We also define the irradiance \( E(r, \Omega_0) = \int_{4\pi} L_p(r, \Omega) d\omega = E_0(r) \exp(-\mu t) \). With this definition, replacing eq. (1.6) in (1.4) and substituting for \( L(r, \Omega) \) in (1.1) one obtains the transport equation for the scattered radiance:

\[
\Omega \cdot \nabla L_s(r, \Omega) + \mu_t L_s(r, \Omega) = \mu_s \int_{4\pi} p(\Omega, \Omega') L_s(r, \Omega') d\omega' + \mu_s p(\Omega, \Omega_0) E(r, \Omega_0),
\]

where we have used (1.5) to cancel the terms related to \( L_p(r, \Omega) \), and the fact that \( \int_{4\pi} p(\Omega, \Omega') \delta(1 - \Omega \cdot \Omega_0)/(2\pi) d\omega = p(\Omega, \Omega_0) \). In effect, we have made the primary radiance the source term in eq. (1.8).

To make progress into solving (1.8), it is practical to expand the angular part of \( L_s(r, \Omega) \) in spherical harmonics\(^3\). The first two terms of this expansion can be written in the form

\[
L_s(r, \Omega) = \frac{1}{4\pi} \int_{4\pi} L_s(r, \Omega) d\omega + \frac{3}{4\pi} \int_{4\pi} L_s(r, \Omega') \Omega' \cdot \Omega d\omega' = \left(\frac{1}{4\pi}\right) \Phi_s(r) + \left(\frac{3}{4\pi}\right) F(r) \cdot \Omega.
\]

(1.9)

Using only these first two terms is called the diffusion approximation for reasons that will become evident in a few moments. Considering more terms in the expansion leads to \( P_n \) approximations, of which \( P_3 \) is the most familiar [Dickey et al. 2001]. Including more terms in the expansion permits a better description of the angular behaviour of the fluence and provides a better approximation to the real phenomenon. However, using more terms requires more free parameters that may not be easily determined from the boundary conditions.

To derive the steady state diffusion equation we need to find a differential equation in terms of \( \Phi(r) \) only (the zeroth-order term in the spherical harmonic expansion). First, let us integrate eq. (1.8) over the \( 4\pi \) solid angle. The integration of the first term can be achieved by recalling that \( \nabla \) only operates on \( r \) and not on \( \Omega \):

\[
\int_{4\pi} \Omega \cdot \nabla L_s(r, \Omega) d\omega = \int_{4\pi} \nabla \cdot (L_s(r, \Omega) \Omega) d\omega = \nabla \cdot (\int_{4\pi} L_s(r, \Omega) \Omega d\omega) = \nabla \cdot F(r).
\]

(1.10)

\(^3\)The reader is referred to any standard text book of mathematical methods for physicists for a detailed description, for example [Arfken & Weber 2005].
Chapter 1. Introduction

Recalling the normalization of the scattering phase function yields an expression of the energy conservation:

$$\nabla \cdot \mathbf{F}(\mathbf{r}) = -\mu_a \Phi_s(\mathbf{r}) + \mu_s E(\mathbf{r}, \Omega_0).$$  \hspace{1cm} (1.11)

This equation says that the rate of change of energy per unit of volume ($\nabla \cdot \mathbf{F}(\mathbf{r})$) is equal to the energy being lost by absorption plus the energy being scattered into the volume from the primary irradiance.

To obtain an expression for $\mathbf{F}(\mathbf{r})$ in terms of $\Phi(\mathbf{r})$ we multiply eq. (1.8) by $\Omega$ and integrate again over the $4\pi$ solid angle. This is when the diffusion approximation is used. The first term after expanding $L_s(\mathbf{r}, \Omega)$ according to (1.9) becomes

$$(1/4\pi) \int_{4\pi} d\omega \Omega (\mathbf{\Omega} \cdot \nabla \Phi_s(\mathbf{r})) + (3/4\pi) \int_{4\pi} d\omega \mathbf{\Omega} [\mathbf{\Omega} \cdot \nabla (\mathbf{F}(\mathbf{r}) \cdot \mathbf{\Omega})] = (1/3) \nabla \Phi_s(\mathbf{r}),$$  \hspace{1cm} (1.12)

where the vector identities $\int_{4\pi} \mathbf{\Omega} (\mathbf{\Omega} \cdot \mathbf{A}) d\omega = (4\pi/3) \mathbf{A}$ for $\mathbf{A}$ independent of $\mathbf{\Omega}$, and $\int_{4\pi} \mathbf{\Omega}[\mathbf{\Omega} \cdot \nabla (\mathbf{A} \cdot \mathbf{\Omega})]d\omega = 0$, for any $\mathbf{A}$. The first term in the right hand side of eq. (1.8) can be calculated by first noticing that

$$\int_{4\pi} p(\mathbf{\Omega} \cdot \mathbf{\Omega}')\mathbf{\Omega} d\omega = \int_{4\pi} p(\mathbf{\Omega} \cdot \mathbf{\Omega}') (\mathbf{\Omega} \cdot \mathbf{\Omega}') \mathbf{\Omega}' d\omega = g \mathbf{\Omega}',$$  \hspace{1cm} (1.13)

where we have called $g$ the average cosine of the phase function and used the fact that by integrating, components that are not along $\mathbf{\Omega}'$ cancel out each other, leaving only the components along $\mathbf{\Omega}'$ given by $(\mathbf{\Omega} \cdot \mathbf{\Omega}') \mathbf{\Omega}'$. After applying once more the first of the two vector identities mentioned above, the term becomes $\mu_s g \mathbf{F}(\mathbf{r})$. Applying once more (1.13) for the last term in eq. (1.8), and putting all the terms together yields

$$(1/3) \nabla \Phi_s(\mathbf{r}) + \mu_t \mathbf{F}(\mathbf{r}) = \mu_s g \mathbf{F}(\mathbf{r}) + \mu_s g E(\mathbf{r}, \Omega_0) \Omega_0$$  \hspace{1cm} (1.14)

or equivalently

$$\mathbf{F}(\mathbf{r}) = -(1/3\mu_{tr}) \nabla \Phi_s(\mathbf{r}) + (\mu_s g / \mu_{tr}) E(\mathbf{r}, \Omega_0) \Omega_0,$$  \hspace{1cm} (1.15)

where $\mu_{tr} = \mu_t - \mu_s g = \mu_a + (1 - g) \mu_s = \mu_a + \mu'_s$ is the transport attenuation coefficient. The diffusion coefficient that appears in the conventional diffusion equation is related to
\( \mu_{tr} \) as \( D = 1/(3\mu_{tr}) \). Finally, substituting for \( F(r) \) from eq. (1.15) into (1.11) gives the inhomogeneous diffusion equation

\[
\nabla^2 \Phi_s(r) - 3\mu_a\mu_{tr} \Phi_s(r) = -3\mu_{tr}\mu_a E(r, \Omega_0) + 3\mu_s g \nabla \cdot (E(r, \Omega_0)\Omega_0) .
\]

(1.16)

It is customary to introduce an effective attenuation coefficient defined as \( \mu_{eff}^2 = 3\mu_a\mu_{tr} \).

### 1.3.2 Solution for an isotropic point source

Let us now solve this equation for an isotropically emitting point source. Once we find \( \Phi(r) \) we only have to add \( \Phi_p(r) \) and we will have the full solution for a point source. Previously we had found in eq. (1.7) that for a point source, \( E(r, \Omega_0) = (1/4\pi) \exp(-\mu_t r)/r^2 \).

Also notice that the unit vector \( \Omega_0 \) is simply the unit vector \( \hat{r} \). Replacing this expression in (1.16) and using spherical coordinates, one obtains

\[
\nabla^2 \Phi(r) - \mu_{eff}^2 \Phi(r) = 3g\mu_s \delta(r) - C \frac{\exp(-\mu_t r)}{r^2} = S_{\text{point}}(r) ,
\]

(1.17)

where \( C = (3/4\pi)\mu_s(g\mu_t + \mu_{tr}) \) is used to collect constant terms. We will use the method of Green’s function to find a particular solution to this differential equation. For this equation (also called a modified Helmholtz’s equation) the Green’s function is \( G(r, r_1) = -(1/4\pi) \exp(-\mu_{eff} ||r - r_1||)/||r - r_1|| \), where the minus sign appears because positive sources in the diffusion equation appear with a minus sign at the right side of the equality.

The solution to the inhomogeneous equation can be formulated as

\[
\Phi(r) = \int G(r, r_1) S_{\text{point}}(r) dr_1^3.
\]

The term containing the delta function can be easily evaluated to be

\[
-3g\mu_s \int (1/4\pi) \frac{\exp(-\mu_{eff} ||r - r_1||)}{||r - r_1||} \delta(r_1) dr_1^3 = -(3/4\pi)g\mu_s \frac{\exp(-\mu_{eff} r)}{r} .
\]

(1.18)
For the second term, first we note that in spherical coordinates $\|\mathbf{r} - \mathbf{r}_1\| = (r^2 + r_1^2 - 2rr_1 \cos \theta)^{1/2}$. We thus have

\[
(-C/4\pi) \int_0^\infty r_1^2 dr_1 \frac{\exp(-\mu_1 r_1)}{r_1^2} \int_0^{2\pi} \int_0^1 d\phi d(\cos \theta) \frac{\exp(-\mu_{\text{eff}} (r^2 + r_1^2 - 2rr_1 \cos \theta)^{1/2})}{(r^2 + r_1^2 - 2rr_1 \cos \theta)^{1/2}}
\]

\[
= (-C/2\mu_{\text{eff}} r) \int_0^\infty dr_1 \frac{\exp(-\mu r_1)}{r_1} [\exp(-\mu_{\text{eff}} \|r - r_1\|) - \exp(-\mu_{\text{eff}} (r + r_1))].
\]

Now, we must make the assumption that $\mu t \gg \mu_{\text{eff}}$ which says that the contribution due to $\exp(-\mu_1 r_1)$ vanishes far from the source. Therefore, we can assume safely that the region where $r_1 < r$ contributes almost solely to the integral. This allow us to replace $\|r - r_1\|$ simply by $r - r_1$. Collecting the term $\exp(-\mu_{\text{eff}} r)$ out of the integral one obtains

\[
-C \frac{\exp(-\mu_{\text{eff}} r)}{2\mu_{\text{eff}} r} \int_\epsilon^\infty dr_1 \frac{1}{r_1} [\exp(-r_1(\mu_t - \mu_{\text{eff}})) - \exp(-r_1(\mu_t + \mu_{\text{eff}}))].
\]

The lower limit of the integral has been replaced by $\epsilon$ since this integral diverges at zero. Noting that near zero, the exponential integral $E_1(x) = \int_x^\infty \exp(-t)/t \approx -\ln x$ [Abramowitz & Stegun 1965], and performing a change of variables for the terms $r_1(\mu_t - \mu_{\text{eff}})$ and $r_1(\mu_t + \mu_{\text{eff}})$ we obtain

\[
-C \frac{\exp(-\mu_{\text{eff}} r)}{2\mu_{\text{eff}} r} [\ln(\mu_t - \mu_{\text{eff}}) + \ln(\mu_t + \mu_{\text{eff}})] = -C \frac{\exp(-\mu_{\text{eff}} r)}{2\mu_{\text{eff}} r} \ln \frac{\mu_t + \mu_{\text{eff}}}{\mu_t - \mu_{\text{eff}}}.
\]

Using the fact that $\mu_t \gg \mu_{\text{eff}}$ and keeping only first order terms in $\mu_{\text{eff}}/\mu_t$, the logarithm can be approximated as $2\mu_{\text{eff}}/\mu_t$. Putting all terms together and adding the primary fluence rate we obtain:

\[
\Phi(r) = \frac{3\mu_s \mu_t}{4\pi \mu_t} \exp\left(-\frac{\mu_{\text{eff}} r}{r}\right) + \frac{1}{4\pi} \frac{\exp(-\mu t r)}{r^2}.
\]  

(1.19)

Note that in the process, the terms corresponding to $3\mu_s g \nabla \cdot (E(r, \Omega_0) \Omega_0)$ in eq. (1.16) have cancelled out.

We can check whether this equation is consistent by ensuring energy conservation. For a point source, $\int d^3 \mu_s \Phi(r) = 1$, which is indeed true for eq. (1.19). By using our previous assumptions that $\mu_t \gg \mu_{\text{eff}}$ and $\mu_s \gg \mu_a$, we can remove the primary fluence
rate and cancel the fraction $\mu_s/\mu_t$ yielding
\[
\Phi(r) = \frac{3\mu_tr \exp(-\mu_{\text{eff}} r)}{4\pi r},
\]
which is the same expression used at the beginning of Chapter 2 (eq. (2.1)), recalling that $\mu_{\text{eff}}^2/\mu_a = 3\mu_tr$. We will further address the validity of this approximation in Chapter 3.

Venugopalan et al. [1998] have addressed the extension of the diffusion equation to highly absorbing media and discuss several boundary conditions. A detailed highly mathematical treatment of the transport equation from the perspective of optical tomography is presented in [Arridge 1999].

1.4 Hypotheses and specific aims

In this dissertation I have explored two major hypotheses with corresponding specific aims:

**Hyp 1:** Tailored cylindrical diffusers improve our ability to conform the light dose to the target geometry, consequently improving the therapeutic index, over conventional diffusers.

**S.Aim A:** To validate the model of light propagation in tissue using Monte Carlo simulations and tissue phantom experiments (Chapter 3).

**S.Aim B:** To develop an optimization algorithm for treatment planning of light delivery for PDT of the prostate (Chapter 2).

**S.Aim C:** To compare the treatment plans using tailored or conventional diffusers and analyze their sensitivity to uncertainties in the parameters of the model (Chapter 2).

**S.Aim D:** To characterize the ability of a tailored diffuser for modulating the contour lines of the fluence rate distribution, in order to understand the
potential to conform to a desired target and its constraints (Chapter 3).

**Hyp 2:** EPO reduces the neuronal toxicity of PDT *in vitro* and reduces the volume of PDT induced necrosis in normal rat brain *in vivo.*

**S.Aim A:** To test whether EPO protects cultured cortical neurons from ALA mediated PDT (Chapter 4).

**S.Aim B:** To test whether EPO reduces the volume of necrosis of Photofrin mediated PDT induced lesions in the normal rat brain 24 h after light delivery (Chapter 4).

## 1.5 Thesis organization

The next two chapters of this dissertation deal with a physical strategy to improve the therapeutic ratio. These two chapters are tightly interconnected; Chapter 3 serves both as a prequel and a sequel to Chapter 2. Chapter 3 goes into detail to understand and verify the assumptions used in Chapter 2 with regards to the model of light propagation in tissue. Once that is done, the chapter presents a formalism to understand to what extent a cylindrical diffuser with customizable emission profiles can shape the light dose distribution. This is used to explain the results of Chapter 2. However, by itself, Chapter 3 constitutes a complete story of the characterization of the forward problem, and as such, it has been previously published ([Rendon et al. 2006](#)).

Chapter 2 presents the main results with respect to treatment planning with tailored diffusers. In addition, it spends a considerable effort introducing and verifying an optimization algorithm to reliably compare treatment plans under different scenarios. This chapter also has two small appendices introducing the concept of the singular value decomposition (section 2.A) and addressing the need for the regularization of inverse problems (section 2.B).
For Chapter 4, I switch gears to studying a biological approach to improve the TI in the treatment of brain tumours with PDT.

Because of the breadth of these approaches, this introduction has been kept fairly general. A more specific treatment of the motivation and current stand of these subjects has been given in the introductions to each chapter, particularly in those of chapters 2 and 4.

The same can be said about the discussion of this dissertation. For clarity, the bulk of the discussion is presented in each chapter. The final chapter will conclude by highlighting the main findings of each chapter, and possible future research avenues. Then I will conclude by attempting to answer what this research has to offer to the future of PDT.
Chapter 2

Treatment planning using tailored light diffusers for PDT of the prostate

Chapter summary

Interstitial Photodynamic therapy (PDT) has seen a rebirth, partially prompted by the advent of photosensitizers with longer absorption wavelengths, which enable the treatment of larger tissue volumes. This chapter addresses the problem of planning light delivery using cylindrical diffusers for PDT of the prostate. In particular, it studies whether using diffusers with customizable longitudinal emission profiles, rather than conventional ones with constant emission profiles, improves our ability to conform the light dose to the target’s geometry.

I present an optimization algorithm to solve the treatment planning problem which improves upon previous approaches in the literature. Based on this algorithm, I compare the treatment plans obtained under a variety of light delivery scenarios using 5-15 standard or tailored diffusers. The sensitivity of the resulting plans to uncertainties in the optical properties and the placement of diffusers is studied.

The study finds that tailored diffusers only marginally outperform conventional ones in terms of prostate coverage and rectum sparing. Furthermore, it is shown that small changes in optical properties can lead to large changes in the light dose distribution, but that those changes can be largely reverted with a simple light dose renormalization. Finally, I find that the placement of the diffusers affects prostate coverage only minimally.

The implications of these results for treatment planning in the context of interstitial PDT are discussed.
2.1 Introduction

Photodynamic therapy (PDT) has become widely accepted for treating a variety of superficial lesions, resulting in good clinical and cosmetic outcomes. It is successfully used for treating superficial cancers of the skin [Moseley et al. 2006], oesophagus [Chen et al. 2006b], upper respiratory tract [Kato et al. 2006, Loewen et al. 2006], head and neck [Biel 2006], and other locations where surface illumination is possible. However, the same success has not been achieved when treating deep-seated tumours that require interstitial light delivery, in part, due to the very steep attenuation of light in this illumination geometry. The development of photosensitizers with absorption bands at longer wavelengths, and therefore greater penetration depths, has prompted a rebirth in the field of interstitial PDT. As a result, several groups have recently initiated clinical trials for interstitial PDT, in particular for prostate cancer [Du et al. 2006, Moore et al. 2006, Trachtenberg et al. 2007].

The most common light delivery strategy for deep-seated tumours is to interstitially place cylindrical light diffusers fibre-coupled to an external laser. Such diffusers are manufactured to emit with constant longitudinal emission profiles. Recently, cylindrical diffuser with customizable longitudinal profiles have become available [Vesselov et al. 2005]. These diffusers should, in principle, enable delivering light dose distributions that match the target volume better than conventional diffusers. It is unclear, however, whether the added flexibility in the emission profile will indeed improve the light dose distribution. This study will focus on determining whether the light dose can be made more conformal in prostate PDT.

Optimization-based treatment planning for interstitial PDT is a nascent field. Treatment planning has been traditionally performed manually, although it is clear that automation would be advantageous. Computer-based optimization can improve planning efficiency and potentially find better solutions, yet only a few groups have investigated such strategies [Altschuler et al. 2005, Johansson et al. 2007a]. These groups have im-
implemented a common optimization strategy, where a search over diffuser positions is combined with a Cimmino linear feasibility algorithm [Censor et al. 1988] to find the output powers (or irradiation times) for the diffusers. As shown here, the Cimmino feasibility algorithm converges to solutions that do not necessarily reflect a desired treatment plan by minimizing the cost function chosen. Consequently, I have modified the Cimmino algorithm in order to also minimize the cost function in the inconsistent case.

Treatment planning requires knowledge of the tissue optical properties to ensure adequate dosimetry. Measuring these properties \textit{in vivo} is not trivial, and it often leads to uncertainties of up to 40% [Trachtenberg et al. 2007]. Furthermore, these values tend to change between the planning and the delivery phases, for example because of variations in blood volume fraction and oxygenation [Jankun et al. 2005, Zhu et al. 2005b, Jiang et al. 2003, Svensson et al. 2007], or photosensitizer concentration [Lilge & Wilson 1998]. Diffuser placement is also subject to uncertainty because of template alignment, diffuser insertion, and changes in the shape of the prostate between imaging and delivery. To our knowledge no study has systematically analyzed the sensitivity of the light dose distribution with respect to changes in these design variables.

This chapter achieves two objectives. Firstly, I establish under what circumstances tailored cylindrical diffusers perform better than conventional ones in PDT of the prostate. To this end, I have implemented an optimization strategy involving a modified Cimmino algorithm that adequately minimizes the cost function of the search process. Secondly, I determine the sensitivity of the planned light dose distributions to uncertainties in the delivery parameters, particularly the optical properties and diffuser placement.
2.2 Methods

2.2.1 Test case geometry and light dosimetry model

Test case

A 34 cc prostate test case was obtained by tracing T2-weighted transverse MRI slices through a prostate of a patient suited for PDT treatment. The model resolution was downsized to $1 \times 1 \times 3$ mm along the $x$, $y$ and $z$-axes respectively. The centre of the urethra was traced and a 5 mm diameter was assumed. Only the anterior surface of the rectum was included in the test geometry (fig. 2.1).

Figure 2.1 – Description of the geometry of the test case. The prostate is indicated in red, the rectum in blue and the urethra in green. The template grid is shown on the $x,y$-plane. The maximum projection of the prostate (and cross sections of the urethra and rectum at the same height) is superimposed on the template. Only rectal surface at positions $y > -1$ cm was considered.
Diffusers are inserted in the prostate, parallel to the $z$-axis, using a brachytherapy template consisting of a square grid of slots, spaced every 5mm. The possible $(x,y)$ positions of the diffusers were confined to the interior of the prostate, represented by the red line on the template shown in Fig. 2.1, with a total of 48 slots available.

**Light dosimetry**

The light dose\(^1\) arising from a cylindrical diffuser is calculated as a convolution of a point source function and the emission profile of the diffuser. The point source function (also called kernel with units of cm\(^{-2}\)), \(G(r)\), is adopted from the standard diffusion theory solution for the fluence arising from a point source emitting isotropically with unit power for unit time in an infinite medium [Arnfield et al. 1989]:

\[
G(r) = \frac{\mu_{\text{eff}}^2}{4\pi \mu_a} \exp \left( -\frac{\mu_{\text{eff}}}{\mu_a} r \right),
\]

where $\mu_a$ and $\mu_{\text{eff}}$ are the absorption and effective attenuation coefficients. This study assumes $\mu_a = 1$ cm\(^{-1}\), thereby assigning $\mu_{\text{eff}}$ as the only relevant tissue optical property.

The emission profile $S(z')$ expresses the emitted energy per unit of length for a diffuser. $S(z')$ is constant for diffusers with flat emission profiles and arbitrary for tailored diffusers. The arbitrary definition of $\mu_a$ makes the source units also arbitrary, and therefore, also the units of light dose\(^2\). By superposition, the light dose arising from diffuser $k$ is expressed as the convolution

\[
\Phi_k(\vec{r}) = \int G(x - x_k, y - y_k, z - z') S_k(z') dz',
\]

---

\(^1\) Light dose is best defined as fluence (J/cm\(^{-2}\)), which can be obtained from the fluence rate (W/cm\(^{-2}\)) by multiplying by the irradiation time, when necessary. This multiplication by time will be omitted from the discussion but implicitly assumed. That, in effect, means that eq. (2.1) is multiplied by a 1 J source.

\(^2\) In this chapter, the threshold model for the biological response to PDT dose is assumed [Patterson et al. 1990]. Furthermore, any reference to photosensitizer concentration is incorporated into the threshold light dose defined in Table 2.1. Therefore, the terms: fluence, light dose, and dose become equivalent.
where \(x_k\) and \(y_k\) specify the position of the diffuser. Diffuser are assumed to remain parallel to the \(z\)-axis. The light dose distribution arising from \(K\) diffusers is obtained as \(\sum_{k=1}^{K} \Phi_k(\vec{r})\).

Equation (2.2) is discretized by sampling evenly the space coordinates \(x\), \(y\), and \(z\), as well as the diffuser coordinate \(z'\). After lexicographic indexing of the spatial coordinates by \(i\) (\(M\) voxels in total), and adding the contributions from all diffusers, eq. (2.2) takes the form

\[
\phi_i = \sum_j G_{ij} s_j, \tag{2.3}
\]

where \(s_j\) includes all \(K\) diffuser profiles concatenated into a single vector \(s\) of length \(N\). The size of matrix \(G\) is therefore \(M\)-by-\(N\).

The accuracy of the discretization was improved by initially sampling \(S(z')\) at 0.5 mm intervals, and then re-sampling \(G\) at 3 mm intervals. This reduces the dimension of the problem and improves the stability of the solutions. The final discretization was 1 mm along the \(x\) and \(y\)-axes, and 3 mm along the \(z\) and \(z'\)-axes.

Observation voxels were selected on the surfaces of the prostate, the rectum and the urethra. Other voxels were taken inside the prostate and in the surrounding tissue (background tissue) to improve the homogeneity of the light dose within the prostate and to avoid delivering large doses to surrounding tissue. All available voxels were used to calculate dose volume histograms (DVH) (see sec. 2.3.2). The number of observation points used for each organ is shown in table 2.1.

### 2.2.2 Solution to the inverse problem

The inverse problem of treatment planning corresponds to finding \(s_j\) given some specification of the light dose \(\phi_i\). The specification used here prescribes a set of upper and lower light doses \(d^U_i\) and \(d^L_i\) for each observation voxel \(i\). These doses correspond to the thresholds of necrosis for the respective organs (assuming a threshold model of PDT tissue response [Patterson et al. 1990]) based on clinical findings at our institution for the
Table 2.1 – Lower and upper dose constraints, and number of observation voxels for each organ. The weight per voxel used in this study is also shown.

<table>
<thead>
<tr>
<th>Organ</th>
<th>$d^L$</th>
<th>$d^U$</th>
<th>voxels</th>
<th>$w_i \times 10^{-4}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>prostate</td>
<td>100</td>
<td>1000</td>
<td>786</td>
<td>3.18</td>
</tr>
<tr>
<td>rectum</td>
<td>0</td>
<td>62.5</td>
<td>277</td>
<td>9.03</td>
</tr>
<tr>
<td>urethra</td>
<td>0</td>
<td>100</td>
<td>65</td>
<td>38.5</td>
</tr>
<tr>
<td>background</td>
<td>0</td>
<td>100</td>
<td>1319</td>
<td>1.90</td>
</tr>
</tbody>
</table>

photosensitizer WST01 with concurrent photosensitizer and light administration, where at least 40 J/cm$^2$ are delivered to the prostate and at most 25 J/cm$^2$ to the rectum [Trachtenberg et al. 2007]. The values normalized to 100 for the prostate are summarized in table 2.1.

Define the residual at each observation point as

$$ r_i(s) = \begin{cases} 
0 & \text{if } d^L_i \leq G_i \cdot s \leq d^U_i \\
(d^L_i - G_i \cdot s) & \text{if } G_i \cdot s \leq d^L_i \\
(d^U_i - G_i \cdot s) & \text{if } d^U_i \leq G_i \cdot s,
\end{cases} \quad (2.4) $$

where $G_i \cdot s$ is the standard inner product between the $i$th row of $G$ and $s$. The inverse problem is solved by minimizing

$$ f(s) = \sum_{i=1}^{M} w_i |r_i(s)|^2 \quad (2.5) $$

subject to $s_j > 0$ for $j = 1, 2, \ldots, N$.

The constraint ensures that all powers remain positive. An optimal solution, $s^*$, is specified by the powers (or emission profiles for tailored diffusers), lengths and positions of the diffusers.

The cost function eq. (2.5) expresses the weighted sum of all square deviations from the prescribed dose range, and it is similar to equation (4) in [Altschuler et al. 2005] except for the squaring of the residual, which is of little consequence in terms of the
location of the minimum. Importance weights are introduced for each organ, where $w_i$ is equal to the weight of the organ it belongs to, divided by the number of observation voxels in that organ. Here, all organs receive equal weights.

We have solved two different problems for each type of diffuser (flat and tailored). (I) The free parameters are the template positions and the powers (or emission profiles) for diffusers that extend across the full length of the prostate, at their given template positions. (II) Diffuser lengths and $z$ locations are also free parameters. The four possible problem scenarios are summarized in table 2.2.

<table>
<thead>
<tr>
<th>Problem Type</th>
<th>Scenario</th>
<th>Diffuser</th>
<th>Design Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1F</td>
<td>Flat</td>
<td>$x, y$; powers</td>
</tr>
<tr>
<td>I</td>
<td>1T</td>
<td>Tailored</td>
<td>$x, y$; power profiles</td>
</tr>
<tr>
<td>II</td>
<td>2F</td>
<td>Flat</td>
<td>$x, y, z$; lengths; powers</td>
</tr>
<tr>
<td>II</td>
<td>2T</td>
<td>Tailored</td>
<td>$x, y, z$; lengths; power profiles</td>
</tr>
</tbody>
</table>

An algorithm with two distinct loops was implemented to minimize eq. (2.5). The outer collection of loops optimizes the lengths and positions of the diffusers, while the inner loop optimizes the powers or power emission profiles. The listing below summarizes the algorithm.
% Outer loops
loop(over 20 starting positions) % INITIALIZATION
   do % until convergence is achieved
      loop(over random permutation of diffusers) % SEARCH OVER TEMPLATE SLOTS
         choose diffuser
         loop(over empty slots)
            move diffuser to slot
            do % LENGTH AND Z POSITION (only for type II problems)
               loop(over random permutation of diffusers)
                  trim/extend diffusers
   % Inner loop returns optimal cost
      cost=feasibility algorithm % modified Cimmino algorithm
         while(no convergence at slot)
            while(no convergence)
               Select minimum cost solution among all starting conditions

### Outer loops

This section consists of three parts: initialization of the diffuser positions, search over the possible positions, and, for type II problems, search over the lengths and z positions of the diffusers. Our implementation is guided by the assumption that, in the vicinity of a diffuser, the light distribution is determined by that diffuser. As a result, one can replace searching exhaustively over all the possible combinations of positions by iteratively searching for a single diffuser while keeping all others fixed. This is an arguable assumption as evidenced by the fact that not all starting conditions converge to the same solution.

**Initialization.** A multi-start approach was implemented to improve the chances of finding a global minimum. For type I problems, optimization was started from twenty
initial, randomly chosen, sets of positions. To speed up type II problems, the 10 sets of positions yielding the lowest costs during the optimization of 1T were used as starting points.

**Search over \(x, y\) positions (template slots).** The algorithm chooses a diffuser randomly while fixing the positions of other diffusers. Then it proceeds to find the minimum cost position for that diffuser over all the available template slots. This is repeated for the other diffusers. The whole process is repeated until the cost converges, with typically 3 to 5 iterations needed.

**Search over lengths and \(z\) positions.** Only type II problems implement this additional search. For each set of template positions a diffuser is chosen randomly and its length is set to fully extend along the length of the prostate. First, the distal end is reduced by 3 mm at a time until the cost no longer decreases. The proximal end is then equally trimmed, followed by extension of the distal end and then of the proximal end. This four-step loop is repeated for that diffuser until the cost ceases to decrease, and then repeated with the remaining diffusers. The whole process is then repeated until cost convergence is obtained.

**Inner loop**

The inner loop implements a feasibility algorithm that attempts to find a solution \(s^*\) that satisfies all the constraints. Since feasible solutions are uncommon because the number of constraints is much larger than the number of design parameters, the algorithm reverts to minimizing eq. (2.5) in a least-square sense. We employ the following linear feasibility algorithm:
Algorithm 1. Choose $\lambda = \rho(G^T W G)^{-1}$ and start with $s^0 = 0$.

Then iterate:

$$\hat{s}^{k+1} = s^k + \lambda \left( \sum_{i=1}^{M} w_i r_i(s^k) G_i \right)$$

(2.6)

$$s^{k+1} = \begin{cases} 
\hat{s}^{k+1}_j & \text{if } \hat{s}^{k+1}_j \geq 0 \\
0 & \text{if } \hat{s}^{k+1}_j < 0 
\end{cases}$$

(2.7)

until $k > 80$ or $\| f(s^{k+1}) - f(s^k) \| < 10^{-3}$.

Here $\rho(A)$ is the spectral norm of matrix $A$ (the largest singular value of $A$), and $W$ is the $M$-by-$M$ matrix with diagonal entries equal to the weights $w_i$. Equation (2.7) ensures that powers emitted at all fibre positions remain positive. The stopping criteria has been empirically found to provide a compromise between low costs and smooth solutions. In fact, algorithm 1 inherently regularizes the problem as a function of iteration number, that is, the less iterations used the smoother the solution obtained. This property of the algorithm is derived in subsec. 2.B.1.

2.2.3 Sensitivity analysis

The sensitivity to changes in optical properties of the light dose distributions for the solutions to all scenarios was analyzed by calculating the fluence rate distribution resulting from changing $\mu_{\text{eff}}$ by $\Delta \mu_{\text{eff}} = \pm 0.3 \text{ cm}^{-1}$. The resulting light dose distributions for these two extremes were compared with the nominal solution. The sensitivity to diffuser placement was also determined. Optimal diffuser positions were perturbed by adding a noise vector in the $x,y$-plane uniformly drawn from $(-0.3, 0.3) \times (-0.3, 0.3)$. 100 such samples were generated.
2.3 Results

2.3.1 Optimization algorithm

Conditioning of the inverse problem

The condition number ($\kappa$, the ratio of the largest to the smallest singular value) quantifies how sensitive the inverse problem is to small changes in the left-hand side of eq. (2.3) (cf. section 2.B). A large $\kappa$ reflects noise amplification, and therefore characterizes unstable inversions. Under our implementation, problems using flat diffusers are well behaved with $\kappa$ between 2 and 6, depending on $\mu_{\text{eff}}$ and on the number of diffusers. In contrast, $\kappa$ is of the order of $10^2$ for problems involving tailored diffusers, and increases further with the number of diffusers. In fact, prior to sampling the diffuser coordinate at 3 mm intervals, we had sampled it at 1 mm (the manufacturer’s resolution), resulting in $\kappa$ of the order of $10^5$. Such condition numbers lead to diffuser profiles that appear very noisy (spiky) and are difficult to manufacture. The beginnings of this noise amplification can be seen in some portions of fig. 2.2, particularly for the first diffuser in the bottom-most plot (15 diffusers), at the centre of the profile.

Convergence

Other groups have proposed using the Cimmino feasibility algorithm [Censor et al. 1988] for the inner loop [Altschuler et al. 2005, Johansson et al. 2007b]. However, De Pierro & Iusem [1985] have shown that, for the inconsistent case, the Cimmino algorithm minimizes

$$\sum_{i}^{M} w_i |r_i(s)|^2 \sum_{j}^{N} |G_{ij}|^2$$  

(2.8)

\[A way to visualize this is to imagine trying to numerically find the inverse of a function such as \(y = f(x) = x^{-2}\) for large \(x\). Very small changes in the value of \(y\) (e.g. noise) lead to very large changes in \(x\).\]
Figure 2.2 – Sample of emission profiles, $s$, obtained by solving scenario 2T with $\mu_{\text{eff}} = 2.5 \text{cm}^{-1}$ for 7, 11, and 15 diffusers (rows). Each box within a row corresponds to a diffuser of up to 4.8 cm in length. The fluence rate maps for these diffusers are shown in fig. 2.5.

rather than eq. (2.5), making the comparison of different diffusers positions in the outer loop inconsistent. The additional weighing term in the cost function leads to non-monotonic behaviour illustrated in fig. 2.3.

Monotonic convergence as a function of iterations is desirable because stopping rules based on cost change or number of iterations can terminate at undesirable local minima or maxima. Figure 2.3 D shows the cost as a function of iterations for several starting points using the Cimmino algorithm. Notice that some curves diverge even from the start (arrow) or soon thereafter. This is not the case with algorithm 1; although, some curves do not converge monotonically the minimum cost solution (fig. 2.3 B). Algorithm 1
exhibits non-monotonic convergence because different inequalities in equations (2.4) and (2.7) become active or inactive with each iteration.

![Convergence curves for different diffuser positions](image)

**Figure 2.3** – Typical convergence curves for different diffuser positions depicting the cost as a function of the number of iterations of the inner loop, with $\mu_{\text{eff}} = 2.5 \text{ cm}^{-1}$ and 11 diffusers. At the optimal set of positions, a diffuser was chosen and moved to an empty slot. This process was randomly repeated to sample the convergence of the feasibility algorithms. Figures (A) and (B) were obtained with **algorithm 1**, (C) and (D) with the Cimmino algorithm. Solutions for tailored diffusers are shown in (A) and (C), and for flat diffusers in (B) and (D).

### 2.3.2 Diffuser comparisons

To determine whether tailored diffusers performed better than standard ones and under which scenarios, we solved the inverse problem for each scenario, with four different optical properties ($\mu_{\text{eff}} = 1.5, 2.5, 3.0, \text{ and } 3.5 \text{ cm}^{-1}$) and 5 to 15 (21 for 1F) diffusers.
First we looked at the differences in the minimum cost achieved. Subsequently, the resulting light dose distribution were compared by looking at the iso-dose contours and the dose volume histograms (DVH)\(^4\).

**Cost versus number of diffusers**

Figure 2.4 A shows the cost for scenario 1F. From the four scenarios, this one is the closest to standard clinical implementations (e.g. [Weersink et al. 2005]). Notice that the cost plateaus after a given number of diffusers that depends on \(\mu_{\text{eff}}\) (around 15 for \(\mu_{\text{eff}} = 3.5 \, \text{cm}^{-1}\) and 19 for \(\mu_{\text{eff}} = 3.5 \, \text{cm}^{-1}\)). Figure 2.4 B shows the cost for the remaining three scenarios. For these scenarios, the cost also seems to plateau as the number of diffusers increases, but not as strongly as for scenario 1F.

Comparing figures 2.4 A and B reveals that there is an improvement going from 1F to the other three scenarios, particularly for low values of \(\mu_{\text{eff}}\). Surprisingly, fig. 2.4 B shows that the cost for scenarios 1T, 2F, and 2T does not differ by much. Furthermore, flat diffusers (2F) have lower costs than tailored diffuser (1T), although scenario 2T still performs better than all others.

**Light dose distributions**

Figure 2.5 compares the light dose distribution arising from the solutions to both type I problems, for 7, 11 and 15 diffusers. Prostate coverage, sparing of the rectum and of the urethra appear similar between tailored and flat diffusers. The 62.5 dose units iso-fluence line crosses deeper into the rectum for scenario 1F. Scenario 1T conforms better to the urethra. Under-dosing of the apex \((z \leq 0.6 \, \text{cm})\) of the prostate remains problematic for both cases. Increasing the number of diffusers does not appear to improve apex coverage, but it does deliver a more homogeneous prostate coverage.

---

\(^4\)A DVH, more properly called a cumulative dose volume histogram, expresses the percentage of the volume of an organ that has received less than a given dose. It is useful for summarizing the volumetric distribution of the light dose.
Figure 2.4 – Comparison of the cost obtained from solving the different scenarios. Each data point corresponds to the minimum from the multiple runs for each scenario and the error bar represents the bottom 75 percentile. (A) shows the cost for scenario 1F (◦) for up to 21 diffusers. (B) shows the remaining scenarios for 5 to 15 diffusers: (○) scenario 1T, (+) scenario 2F, and (×) scenario 2T. The colours correspond to the four different $\mu_{\text{eff}}$ used. From top to bottom: 3.5, 3.0, 2.5, and 1.5 cm$^{-1}$. The costs for the different scenarios in (B) have been slightly offset for better comparison.

Results for type II problems are very similar to those for scenario 1T, in agreement with the similarities in the cost. However, because the length of the diffuser is also optimized, some diffusers end up covering only small fractions of the length of the prostate (see for example 2.2 for seven diffusers; diffusers 3 and 4).

In our test case, only the rectum most adjacent to the prostate is over-dosed ($z$ between 1.2 and 1.8 cm). Furthermore, sparing seems to improve with increasing $\mu_{\text{eff}}$ (see fig. 2.6). This is expected since larger $\mu_{\text{eff}}$ lead to light penetrating less into surrounding structures.
Figure 2.5 – Light dose maps for scenarios 1F and 1T for different numbers of diffusers, as labelled in the figures. The red line corresponds to the prostate, the green to the urethra, and the blue to the rectum. The black lines are contour levels at 100 (inner curve) and 62.5 (outer curve) dose units. The light dose is indicated on the vertical colour bars in logarithmic units. $\mu_{\text{eff}} = 2.5 \text{ cm}^{-1}$. 
Figure 2.5 – (Cont.) Light dose maps for scenarios 1F and 1T
Figure 2.5 – (Cont.) Light dose maps for scenarios 1F and 1T
Figure 2.6 – Light dose maps for scenario 1T for different $\mu_{eff}$, as indicated. Solutions for 13 diffusers are shown. See fig. 2.5 for detailed annotation.
Figure 2.6 – (Cont.) Light dose maps for scenario 1T for different $\mu_{\text{eff}}$
**DVH base comparison**

The volumetric distributions of the light dose for the prostate, rectum and urethra are summarized in fig. 2.7 using DVHs. As shown, the main impact of increasing the number of diffusers is to improve prostate coverage without over-dosing more rectum. Also, the volume of over-dosed urethra decreases as more diffusers are used, but overall, the urethra receives larger doses as more diffusers are employed.

In terms of the different scenarios, DVHs for 1T are better than 1F, while further improvement for type II problems is minimal. Scenario 1F performs worse than the other ones in terms of prostate coverage and rectal over-dose, particularly for $\mu_{\text{eff}} = 1.5$ cm$^{-1}$. This is consistent with the early plateauing of the cost for scenario 1F relative to the other scenarios (fig. 2.4). Under scenario 1F, the urethra receives on average lower doses. However, as shown in fig. 2.5, this is at the cost of prostate coverage.

**Dose conformality and uniformity**

Coverage of the prostate and over-dosing of critical organs can be combined into a single metric by scaling the diffuser output powers by a single multiplier, such that 95% of the prostate receives 100 dose units. This metric measures how conformal the delivery is, directly reflecting the therapeutic index. Dose scaling in effect transfers prostate under-dosing to over-dosing of the organ at risk of interest. In medical physics, this procedure is termed dose re-normalization [Lind et al. 1999].

The re-normalized DVHs for the rectum and urethra are shown in fig. 2.8. Although scenario 1T performs marginally better than the other scenarios, improvement is only evident with respect to 1F. This is contrary to what was anticipated from the cost comparison (fig. 2.4), where solutions to scenario 1T were at most equivalent to those obtained for scenario 2F, and always inferior to those of 2T.

For all scenarios rectal over-dosing decreases by about 30% for every 5 additional diffusers used. For the urethra, in contrast, increasing the number of diffusers only has
Figure 2.7 – Comparison of the DVHs for prostate, rectum, and urethra for all scenarios: 1F (···), 1T (—), 2F (−·−), and 2T (−−). The optical properties and number of diffusers are indicated. The relative positions of the curves for the two values of $\mu_{eff}$ are consistent across the rows (number of diffusers). Curves for other values of $\mu_{eff}$ in the range 1.5–3.5 cm$^{-1}$ fall between these two sets of lines. The vertical lines indicate the target minimum dose for the prostate and the maximum permissible doses for rectum and urethra.

A positive effect for large values of $\mu_{eff}$. For scenario 1F with $\mu_{eff} = 1.5$ cm$^{-1}$, there is no improvement in the rectal over-dose as the number of diffusers increases, in agreement with the plateauing of the cost. For this scenario as well, increasing the number of diffusers does not decrease urethral over-dose with $\mu_{eff} = 3.5$ cm$^{-1}$.
Dose uniformity is calculated as the fraction of prostate receiving more than 500 dose units after re-normalization (fig. 2.8). A 50% reduction is observed for every 5 extra diffusers employed. As expected, smaller $\mu_{\text{eff}}$ offer better dose uniformity, which potentially lead to more efficacious treatments as lower fluence rates are encountered by the majority of the organ.

**Figure 2.8** – Measures comparing the conformality and homogeneity of the solutions to scenarios: $1F$ $\diamond (\cdots)$, $1T$ $\circ (-)$, $2F$ $+(-\cdots)$, and $2T$ $\times (-\cdots)$, as a function of the number of diffusers and two different optical properties. Doses have been re-normalized so that 95% of the prostate receives 100 units of dose. Shown from left to right after re-normalization are percent volumes of: (A) rectum receiving more than 62.5 dose units, (B) of urethra receiving more than 100 dose units, and (C) of prostate receiving more than 500 dose units. Curves for other values of $\mu_{\text{eff}}$ in the range 1.5–3.5 cm$^{-1}$ fall between the two sets of lines for each figure.


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2.3.3 Sensitivity analysis

Next, we investigated the sensitivity of the solutions to uncertainties in the model parameters. To study the effects of changes in the optical properties between treatment planning and delivery, light doses were calculated with $\mu_{\text{eff}}$ different from the one used in the optimization (with $\Delta \mu_{\text{eff}} = \pm 0.3 \text{ cm}^{-1}$). This uncertainty constitutes a minimum bound on the error associated with determining $\mu_{\text{eff}}$ in vivo. The effects of diffuser misplacement were modelled by perturbing the diffuser $x, y$-positions randomly, by up to 3 mm.

Changes in optical properties

The light dose distribution is extremely sensitive to changes in the optical properties. This is shown in fig. 2.9 A, where a change of only $0.3 \text{ cm}^{-1}$ alters the prostate coverage by about 15% with respect to the nominal value. Reducing $\mu_{\text{eff}}$ results in more overdosing to the rectum, which is independent of the number of diffusers (fig. 2.9(B)). These results also hold for the other optical properties not shown in fig. 2.9.

Importantly, when dose is re-normalized to ensure adequate prostate coverage, rectal over-dosing does not differ significantly from the nominal case (fig. 2.9 C). This may imply that the shape of the light dose distribution does not vary much; instead, it is only the absolute values of the light dose that changes. The same does not hold for the urethra. While re-normalization partially recovers the nominal distribution, the values fluctuate much more than for the rectum (not shown). Since the DVH for the urethra falls very steeply close to the threshold dose (fig. 2.7), small variations of the light dose correspond drastically change the volume over-dosed. On average, 80% of the urethral volume is over-dosed after re-normalization.
Figure 2.9 – Sensitivity of the fluence rate distribution to changes in $\mu_{\text{eff}}$ of $\Delta \mu_{\text{eff}} = \pm 0.3$ cm$^{-1}$ for all scenarios: 1F $\diamondsuit(\cdots)$, 1T $\circ(\cdots)$, 2F $\pm(\cdots)$, and 2T $\times(\cdots)$. Only $\mu_{\text{eff}} = 2.5$ cm$^{-1}$ is shown for 5 to 15 diffusers. Three measures are shown: (A) percent prostate receiving dose above 100 units; (B) percent rectum receiving dose above 62.5 units; and (C) percent rectum receiving a re-normalized dose above 62.5 units. The nominal solutions are in colour and unlabelled with symbols while the two extreme solutions are labelled and in black. Dose normalization makes difficult to distinguish the nominal and maximum values in (C).

Diffuser placement

Figure 2.10 shows a comparison between the nominal solution and the average of the 25% top and bottom tails of the distribution of simulated diffuser misplacements. The results of this simulation are largely insensitive to the type or number of diffusers. The other optical properties not shown in fig. 2.10 behave similarly to the one depicted, with curves in (B) and (C) slightly shifted down as $\mu_{\text{eff}}$ increases.

Remarkably, fig. 2.10A shows that a large percentage of diffuser shifts do not affect prostate coverage significantly, suggesting that many nearby local minima exist, or alternatively, that the cost function eq. (2.5) is shallow near the optimum.
Figure 2.10 – Sensitivity of the fluence rate distribution to displacements of the diffusers in the $x,y$-plane of up to $\pm3$ mm in both directions, as a function of number of diffusers, for $\mu_{\text{eff}} = 2.5$ cm$^{-1}$. The sensitivity of three measures is shown: (A) percent prostate receiving dose above 100 units; (B) percent rectum receiving dose above 62.5 units; and (C) percent rectum receiving re-normalized dose above 62.5 units. In (A) the upper set of lines corresponds to the nominal solution and the other two sets are the average best and worst cases (see text for details). The nominal solutions in (B) and (C) correspond to the centre lines, the worst scenarios are above and the best ones below the centre line. Solutions to scenarios $1F \diamond (\cdots)$, $1T \circ (\cdots)$, $2F + (\cdots)$, and $2T \times (\cdots)$ are shown. Best and worst cases are in black with corresponding line styles and symbols, while nominal values are in colour but with no symbol.

Rectal over-dose is sensitive to placement errors in a different way. This organ is easily overdosed by placing a diffuser closer than planned. In this sense, rectal over-dose differs from prostate coverage in that it does not require the concerted effort of all diffusers. Figure 2.10(B) shows that rectal over-dose can be better or worse than the nominal solution. Re-normalizing (fig. 2.10 C) reveals that solutions with better conformality exist. Such solutions are possible because the resolution of diffuser placement in this
simulation (1 mm) is finer than for the template (5 mm). In contrast, the dose distribution at the urethra is on average deteriorated with respect to the nominal case (not shown).

**Robust optimization**

A common approach to deal with parameter uncertainty in the optimization process is to implement a robust methodology [Ben-Tal & Nemirovski 2002]. The nominal optimization problem is expanded to include the expected parameter variation into the cost function (see for example [Chan et al. 2006]). This was implemented for dealing with diffuser placement variations along the $x,y$-plane, under scenario 1T. The inner loop was modified to find a solution that minimizes the average cost of the 8 neighbouring 1 mm displacements of each diffuser. The nominal position was assigned half of the probability, the four nearest neighbours received 2/6, and the four corners the remaining 1/6. This probability distribution attempts to describe that half the time the diffuser is placed at the nominal position, but that deviations are equally likely.

This robust implementation did not reduce the sensitivity to diffuser placement in the $x,y$-plane (data not shown). Robust solutions had typically higher output powers and therefore resulted in better prostate coverage. They were indeed more robust in terms of prostate coverage, with best case scenarios overlapping with the nominal case, particularly for larger $\mu_{\text{eff}}$. However, improved coverage was at the cost of greater rectal over-dose. After renormalization, the robust implementation still performed worse than the other solutions in terms of rectal damage.

**2.4 Discussion**

The main goal of this study was to determine whether using tailored diffusers can improve light delivery for interstitial PDT, particularly for prostate treatment. This was carried out by comparing the light dose distributions resulting from four different treatment sce-
narios (table 2.2). Under each scenario, the light dose was optimized to best conform to a prescribed set of constraints. The effects of variations of the optical properties or the placement of the diffusers on the light dose distributions were also studied. Overall, tailored diffusers under scenario 1T performed as well as or better than tailored or standard diffusers under the other delivery scenarios. However, the improvement offered by scenario 1T will likely be masked by the effects of the variations of optical properties found in clinical practice. In contrast, we find that small variations in diffuser placement have little effect in the resulting light dose distribution, particularly in terms of prostate coverage.

2.4.1 Optimization strategy

Altschuler et al. [2005] introduced the Cimmino feasibility algorithm to find the optimal diffuser powers in interstitial PDT. Johansson et al. [2007b] also implemented the Cimmino algorithm for their treatment planning and on-line dosimetry system. Feasibility algorithms are attractive to the treatment planner because, besides being computationally fast, the dose prescription can be easily implemented into the optimization framework.

Initially, we implemented Cimmino’s algorithm but found that, for large $\mu_{\text{eff}}$ the algorithm was not minimizing the cost, as evidenced by the cost worsening as the number of diffusers increased. The reason is that Cimmino’s algorithm minimizes eq. (2.8) rather than the true cost (eq. (2.5)) [De Pierro & Iusem 1985]. Based on results by Jiang & Wang [2003] for linear systems of equations (rather than for linear systems of inequalities required for the feasibility problem), we modified the Cimmino algorithm to minimize eq. (2.5) in the infeasible case. While experimental evidence (fig. 2.3) suggest that algorithm 1 does minimize eq. (2.5), to our knowledge this has not been mathematically proven.

Algorithm 1 is also designed to have regularization properties that result in smooth solutions (cf. subsec. 2.B.1). Such solutions are desired because they are easier to man-
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Before using feasibility type algorithms, a simulated annealing algorithm was used to find the diffuser powers. For reasons explained in section 2.B this approach led to very noisy diffuser profiles [Rendon et al. 2007]. Algorithm 1 not only corrected this, but also offered a parameter to adjust the degree of smoothness desired, namely the number of iterations. Moreover, while algorithm 1 has been designed to minimize eq. (2.5), similar modifications can be made to this class of algorithms (also called pre-conditioned Landweber schemes [Jiang & Wang 2003]) to account for other cost functions.

Algorithm 1 was used to optimize diffuser powers (or emission profiles) while an outer loop searched over the diffuser positions. Before choosing the one diffuser exhaustive search for the outer loop, several other search strategies were tested. These included local down-hill searches (where only lower cost neighbouring positions are chosen) and simulated annealing (where a higher cost neighbouring position can be chosen with non-zero probability). These approaches tended to get stuck in local minima even despite using slow cooling schedules in the simulated annealing. This was partially mitigated by using a variable size neighbourhood that expanded after a certain number of failed attempts. The final outer loop implemented is a down-hill search algorithm where the neighbourhood consists of all available diffuser positions. In relation to the more sophisticated algorithms, this one delivered the best compromise between CPU time, quality of the minima found, and spread of the minima for multiple starting positions.

Overall, we find that our algorithm is slower than the one reported by Altschuler et al. [2005]. On an Intel Core 2 Duo (2 GHz) computer with 2 Gb RAM scenarios 1F and 1T take approximately 150 and 180 seconds respectively (for 12 diffusers and with $\mu_{\text{eff}} = 2.5 \text{ cm}^{-1}$). Type II problems require much longer computation times because of the additional search step. Our goal, however, was not to find a fast algorithm but one that would likely find a global minimum, or alternatively, that would have a small spread of optimal costs. This was necessary to reliably compare solutions for the different
scenarios studied. Judging from the smooth shape of the cost as a function of the number of diffusers (fig. 2.4), this goal was achieved.

2.4.2 Tailored versus Standard diffuser

Altschuler et al. [2005] compared different light delivery strategies for PDT of the prostate using standard diffusers. The authors optimized treatment plans for strategies ranging from fixed diffuser lengths and placement, to our scenario 2F. They found that optimizing more delivery parameters (i.e., individual diffuser powers, diffuser positions, and lengths) improved the light dose distribution in terms of prostate coverage, and urethral and rectal sparing. I have proposed here that using tailored diffusers makes the light dose conform to the prostate better, thereby improving the selectivity of the treatment. Here, the work of Altschuler et al. [2005] is extended to compare flat and tailored diffusers under their best light delivery strategies, called in this chapter type I and type II problems.

Scenario 1T performs better than scenario 1F for all optical properties investigated, particularly for small $\mu_{\text{eff}}$ (figures 2.7 and 2.8). Besides achieving lower costs, the major improvements were in terms of prostate coverage and rectal sparing. This suggests that using tailored diffusers does represent an advantage over standard ones, specially because scenario 1F is already an improvement over what is done in the clinic. However, once the lengths and positions along the z-axis are also optimized (type II problems), the advantage of using tailored diffusers is less clear. Interestingly, scenario 1T seems to marginally outperform both 2F and 2T scenarios after dose re-normalization is performed (fig. 2.8).

Tailored diffusers modulate the light dose perpendicularly to their long axis. Moreover, as I will established in Chapter 3, this spatial modulation is lost rapidly as a function of distance from the diffuser. However, the prostate and the organs at risk run parallel to the z-axis, and do not display a complex profile along this axis. Therefore,
any improvement seen with tailored diffusers likely affects only small volumes that are averaged out in a DVH.

**Number of diffusers**

Regardless of the diffuser type, the best way to improve the dose distribution is to increase the number of diffusers (fig. 2.4). Using more diffusers results in better prostate coverage without compromising rectal sparing (fig. 2.7). In fact, fig. 2.8 A shows that for every five additional diffusers, the volume of rectum over-dosed seems to halve consistently for all optical properties investigated.

Improvement seems to plateau as the number of diffusers increases (fig. 2.4). This is particularly evident for scenario 1F with $\mu_{\text{eff}} = 1.5 \text{ cm}^{-1}$ where no improvement is observed after 7 diffusers. Figure 2.4 A also shows that the plateauing starts later (more diffusers) for larger $\mu_{\text{eff}}$, and that, as more diffusers are used, similar costs can be achieved irrespective of $\mu_{\text{eff}}$. Plateauing seems also to be present with scenarios 1T, 2F, and 2T, albeit to a lesser extent.

Using more diffusers delivers a more uniform light distribution (fig. 2.8 C). With $\mu_{\text{eff}} = 3.5 \text{ cm}^{-1}$, every five additional diffusers halve the volume of prostate receiving more than 5 times the minimum prescribed dose. Better dose uniformity implies that fluence rates throughout the tissue are reduced, reducing oxygen consumption and thus improving PDT efficacy [Henderson *et al.* 2006].

**2.4.3 Threshold model and performance metrics**

This work assumes a threshold model for the biological response to PDT dose. This model ascertains that tissue necrosis occurs above a threshold dose, with no tissue toxicity seen below this dose [Patterson *et al.* 1990]. Evidence for this model in prostate PDT largely depends on the photosensitizer used. Martin & Hahn [2004] have reviewed pre-clinical studies on dogs and early clinical results. They conclude that most studies do find a
well demarcated and reproducible boundary of necrosis. Some photosensitizers such as ALA [Zaak et al. 2003] and Motexium Lutetium [Hsi et al. 2001] do not show a clear relationship between depth of necrosis and light dose. Others such as SnET₂ [Selman et al. 2001] and WST09 [Chen et al. 2002b] do support a threshold model.

With these caveats in mind, the DVH-based performance metrics were used. Prostate coverage has been defined as the volume of the prostate receiving more than the threshold dose for that organ. Based on preliminary clinical results by Trachtenberg et al. [2007] with WST09, good clinical response, in terms of negative biopsies, is seen with more than 95% coverage.

Mild urethral toxicity has been commonly observed in PDT clinical trials. Nevertheless, it has been managed well and has resolved promptly without long term complications [Du et al. 2006, Trachtenberg et al. 2007]. These observations are consistent with earlier pre-clinical studies in dogs [Chen et al. 2002b, Hsi et al. 2001]. As a result, the NTCP of the urethra with PDT seems low, and therefore we have chosen to exclusively focus on the rectum.

Rectal complications are potentially more severe than urethral ones, particularly because necrosis of the rectal wall can easily result in a fistula. Although using the percent rectum over-dosed is a useful metric to compare different delivery scenarios, its relation with the rectal NTCP is more subtle. Firstly, the percent volume over-dosed depends on the total rectal volume defined in the test case, but the total volume at risk that needs to be included is difficult to determine. Secondly, the percent volume overdosed reported by a DVH does not need to be contiguous, and yet over-dosing a small volume can be sufficient to result in complications. In the absence of clinical data to understand the relationship between rectal NTCP and the PDT dose distribution, the metric used here seems a good starting point. Particularly, given that PDT is starkly a local treatment and therefore over-dosed volumes are likely to be contiguous. Rectal over-dose after dose re-normalization is used here as a measure of the therapeutic ratio. Given what was
mentioned above, it might not be an accurate estimate of the therapeutic ratio, but it does have the advantage of combining prostatic coverage (TCP) and rectal over-dosing (NTCP) in a single number.

### 2.4.4 Uncertainties in \( \mu_{\text{eff}} \) and diffuser misplacement

Several groups have measured the optical properties of the prostate, both in animals and humans. Reports coincide in finding sizable variations of the optical properties within the prostate and among different subjects (see for example [Muschter 2003], [Martin & Hahn 2004] and more recently [Svensson et al. 2007]). Furthermore, light delivery has been shown to affect the optical properties at some wavelengths (630 nm [Chen et al. 1997] and 670 nm [Ballangrud et al. 1997]), but not at longer ones (732 nm [Zhu et al. 2003] and 763 nm [Chen et al. 2002b]). These data suggest that oxygen consumption and total hemoglobin concentration play a big role in this phenomenon since haemoglobin displays strong absorption in the 600 to 700 nm region. Because of the observed inherent variability, on-line dosimetry has been proposed by several groups including [Zhu et al. 2005b] and [Jankun et al. 2004]. This variability implies that pre-treatment planning may be limited, since most likely, optical properties encountered during light delivery will differ from those used for treatment planning.

The sensitivity analysis performed here reveals that, if optical properties are assumed to vary homogeneously, only the absolute values of the fluence rate vary, but the shape of the light dose distribution remains nearly constant (fig. 2.9 C). As a result, not only can pre-treatment planning provide an optimal starting condition for light delivery, but also, variations in the optical properties can be dealt with during light delivery, thereby recovering the planned light dose distribution. These findings still need to be corroborated by directly comparing the light dose distributions.

An additional parameter studied was diffuser misplacement. Prostate coverage was found quite robust in terms of diffuser displacement in the \( x,y \)-axis (fig. 2.10 A). On
average, prostate coverage for the top 25 percentile of the perturbed solutions is at most
5% less than for the nominal solution. This means that placing diffusers accurately
during light delivery is not as critical as expected. Furthermore, it suggests that finding
a global optimum is not only very difficult but also not necessary, since many nearby
diffuser positions lead to practically the same dose distribution.

Recently, Johansson et al. [2007b] have tested the sensitivity to changes in optical
properties with their on-line dosimetry system. Assuming a homogeneous change in the
optical properties and calculating new irradiation times for their treatment fibres using
a Cimmino algorithm, they find that prostate coverage can be recovered to near nominal
values. This is in agreement with our findings

Assuming homogeneous changes in the optical properties is too restrictive. As more
clinical data becomes available it would be very interesting to account for variations
in the optical properties in the optimization process, which resemble the actual hetero-
geneity observed. Traditionally, finite element methods are the only algorithms capable
of accounting for heterogeneity. However, they are computationally too demanding to
solve the inverse problem in reasonable times. Li & Zhu [2007] have began investigating
convolution based methods, such as the one used in this work, to tackle this problem.
They propose an alternative kernel derived from the solution of the diffusion equation
for spherical shells of different optical properties, and show predictions for the light dose
distribution better than those that use kernels that assume homogeneity. Much work
remains to be done in this regard, but it is clear that dosimetry for interstitial PDT
would profit extensively from fast and accurate methods that account for heterogeneous
optical properties.

Uncertainty in other parameters was not studied. It would be of special interest to
understand the effect of changes in prescribed threshold doses. However, in the absence
of adequate models relating the TCP and NTCP to the light dose, interpreting results
from such variations is difficult. In any case, this is certainly something that has to be addressed in the future as more clinical data becomes available.

In conclusion, our results suggest that prostate PDT does not profit significantly from using tailored diffusers. Other locations with more complicated geometries, such as tumours of the head and neck that require interstitial light delivery, still remain interesting candidates to test whether tailored diffusers can deliver more conformal light doses.

**Appendix 2.A  Singular value decomposition**

Singular value decomposition (SVD) is a very powerful technique of functional analysis and linear algebra. This appendix is added for the reader unfamiliar with it, and only the main results are summarized here. A more detailed treatment can be found in standard texts; see for example [Trefethen 1997].

The SVD of any arbitrary (here real) matrix $A$ of size $m$-by-$n$ expresses $A$ as the product of three matrices, two orthonormal matrices $U$ ($m$-by-$m$) and $V$ ($n$-by-$n$), and a $m$-by-$n$ matrix $\Sigma$ that has non-negative diagonal entries and zeros elsewhere; i.e.

$$A = U \Sigma V^T. \tag{2.9}$$

The diagonal elements of $\Sigma$ are arranged in decreasing order: $\sigma_1 > \sigma_2 > ... > \sigma_r > 0$ and $\sigma_{r+1} = ... \sigma_{\min(m,n)} = 0$, where $r$ is the rank of the matrix.

This decomposition diagonalizes $A$ in the sense that

$$A v_i = \sigma_i u_i \tag{2.10}$$

and

$$A^T u_i = \sigma_i v_i. \tag{2.11}$$
The $u_i$ and $v_i$ are the columns of $U$ and $V$, and are called the left-, respectively, right-singular vectors of $A$. These provide orthonormal bases for the column and row spaces of $A$.

Such a decomposition always exists and is unique up to paired sign changes in corresponding vectors in $U$ and $V$. The existence is derived from the eigenvalue decomposition of the matrices $AA^T$ and $A^TA$. In fact, the eigenvectors of these matrices correspond to the $u_i$ and $v_i$, respectively, and their eigenvalues are the $\sigma_i^2$.

A geometrical interpretation goes as follows. Given orthonormal basis vectors for the row and column spaces of $A$, the SVD decomposition makes explicit what happens when $A$ or $A^T$ is applied to these vectors. Equations (2.10) and (2.11) specifies by how much the basis vectors stretch or shrink ($\sigma_i$). Remarkably, it does so without mixing the basis vectors (notice only one index is involved); i.e., it diagonalizes $A$.

**Appendix 2.B Regularization of ill-posed problems**

This appendix introduces some aspect of the origin of ill-posed problems and their solution via regularization. It finishes by showing how a simpler version of algorithm 1 achieves regularization.

Equations (2.2) and (2.3) are well known to represent ill-posed problems where: (a) the singular values of $A$ decay gradually to zero; and (b) the ratio of the largest and smallest singular values (the condition number) is large (this problems are also called ill-conditioned). Additionally, the right-singular vectors associated with the smaller singular values tend to have many zero-crossings. As a result, in the presence of noise, the inversion process is unstable leading to large oscillations in the solution. Even without noise, round-off errors can ruin the inversion. Ill-conditioned problems can still be adequately solved using regularization techniques (see for example Neumaier [1998]). This is particularly relevant for finding the emission profiles of tailored diffusers because the large size of the...
matrix $G$ implies very large condition numbers. Finally, the problems associated with ill-conditioning also appear in the linear feasibility approach used here.

Measurement problems usually lead to ill-posed problems. In this context it is easy to provide an intuitive explanations about the origin and the consequences of ill-conditioning. Assume we want to determine a state $x$ from measured data $b$ that is perturbed by zero-mean noise $\eta$. Furthermore, assume that the measuring process is linear and therefore can be expressed as

$$Ax + \eta = b.$$  \hspace{1cm} (2.12)

Typically there are more data points than free parameters ($m > n$), i.e., $\text{rank}(A) \leq n$. These linear systems commonly arise as a consequence of using the superposition principle. For example, in medical imaging $x$ is the anatomy that is “imaged” via $A$ and leads to measured data $b$. Equation (2.3) provides an alternative meaning to this expression in terms of the dosimetry problem. The standard approach to solve inverse problems is to find an $x^*$ that minimizes

$$E = \|Ax^* - b\|^2 = \|Ax^* - (Ax + \eta)\|^2 = \|A(x^* - x) + \eta\|^2. \hspace{1cm} (2.13)$$

The SVD of $A$ is particularly informative about the nature of the solutions to eq. (2.13). The following expansions are a consequence of decomposition eq. (2.9):

$$x = \sum_{j} v_j \cdot x v_j = \sum_{j} \alpha_j v_j, \hspace{1cm} (2.14)$$

$$x^* = \sum_{j} v_j \cdot x^* v_j = \sum_{j} \alpha_j^* v_j, \hspace{1cm} (2.15)$$

$$\eta = \sum_{i} u_i \cdot \eta u_i = \sum_{i} \gamma_i u_i, \hspace{1cm} (2.16)$$
Substituting these expansions in the last term of eq. (2.13), using eq. (2.10), and the orthonormality of the $u_i$ leads to the following expression:

$$E = \left\| A \sum_j^n (\alpha_j^* - \alpha_j)v_i + \sum_i^m \gamma_i u_i \right\|^2 = \sum_{i=1}^r [\sigma_i (\alpha_i^* - \alpha_i) + \gamma_i]^2 + \sum_{i=r+1}^m \gamma_i^2. \quad (2.17)$$

Notice in the second line that the first sum is only performed up to the rank of the matrix and all other components are lost.

Now, a solution that minimizes $E$ must satisfy the following partial derivative equations:

$$\frac{\partial E}{\partial \alpha_i^*} = 0; \text{ for } i = 1, 2, \ldots, n. \quad (2.18)$$

Performing these derivatives leads to the solution

$$\alpha_i^* = \alpha_i + \frac{\gamma_i}{\sigma_i} \quad \text{for } i = 1, 2, \ldots, r, \quad (2.19)$$

$$\alpha_i^* = \text{not identified} \quad \text{for } i = r + 1, \ldots, n. \quad (2.20)$$

Notice first that if $r < n$, the components $\alpha_{r+1}^*$ to $\alpha_n^*$ of $x$ remain unspecified; i.e. components in the null space of $A$ do not change the value of $E$ and are perceived as noise in $x^*$. These components can acquire non-zero values due to round of errors or to the choice of initial conditions. Furthermore, random sampling algorithms such as simulated annealing will invariably return solutions plagued with these null space components.

A full rank $A$ does not solve the whole problem. The second term in eq. (2.19) becomes arbitrarily large if very small singular values are present; recall that this is precisely a condition for being ill posed. Therefore, in the presence of noise, these components dominate the solution, making the inversion process unstable in the sense that $\|x^*\|$ grows unbounded.

Tikhonov [1963] proposed to substitute minimizing eq. (2.13) by minimizing a compromise between the two norms in question, i.e. to minimize

$$\|Ax - b\|^2 + \lambda^2 \|x\|^2 \quad (2.21)$$
instead. \( \lambda \) is called the regularization parameters and expresses the desired degree of smoothness of \( x^* \).

Following the same argument that led to equations (2.19) and (2.20), one obtains for eq. (2.21)

\[
\alpha_i^* = \alpha_i \frac{\sigma_i^2}{\sigma_i^2 + \lambda^2} + \gamma \frac{\sigma_i}{\sigma_i^2 + \lambda^2} \quad \text{for } i = 1, 2, \ldots, r, \quad (2.22)
\]

\[
\alpha_i^* = 0 \quad \text{for } i = r + 1, \ldots, n. \quad (2.23)
\]

Two things have been achieved. Not only have the null space components been suppressed, but a way of suppressing the noise term by adjusting \( \lambda \) is also found. However, notice that increasing \( \lambda \) degrades the solution by a factor \( \sigma_i^2 / (\sigma_i^2 + \lambda^2) \).

### 2.B.1 Regularization properties of iterative algorithms

Iterative solutions to eq. (2.13) also lead to regularized solutions, but instead, the regularization parameter becomes the number of iterations \( k \). Iterative algorithms are preferred for large systems because they do not require costly matrix inversions. To explain why iterative solutions intrinsically regularize, let us see what algorithm 1 does, in terms of its SVD, when it is used to solve eq. (2.12).

First, define \( A = W_1 A, b = W_1 d \), and substitute \( s \) by \( x \). In matrix notation, algorithm 1 takes the form:

**Algorithm 2.** Start with \( x^0 = 0 \), iterate:

\[
x^{k+1} = x^k + \lambda A^T (b - Ax),
\]

This type of iterative algorithm is called a Landweber iteration scheme. Hanke [1991] presents an extensive description of the convergence properties of the general algorithm using a polynomial formalism. Here, we determine the convergence rate using the SVD, that, although less general, provides a more intuitive understanding of the regularization
properties. The critical ingredient to ensure and speed convergence is the particular choice of $\lambda = \rho(A^T A)^{-1} = \sigma_i^{-2}$.

In addition to equations (2.14)–(2.16), consider the expansion $b = \sum_i \beta_i u_i$. Premultiplying eq. (2.24) by $A$, expanding in its SVD, and considering individual components associated with each $u_i$ leads to

$$\alpha^{k+1}_i = \alpha^k_i \left(1 - \frac{\sigma^2_i}{\sigma^2_1}\right) + \frac{\sigma_i \beta_i}{\sigma^2_1}.$$  \hfill (2.25)

We are after the speed of convergence of different components $i$. Subtracting the SVD solution to eq. (2.13) $\alpha^*_i = \beta_i / \sigma_i$ from both sides of eq. (2.25) and taking the norm leaves us with the recurrence relation

$$\|\alpha^{k+1}_i - \alpha^*_i\| = \|\alpha^k_i - \alpha^*_i\| \left(1 - \frac{\sigma^2_i}{\sigma^2_1}\right).$$  \hfill (2.26)

Applying this relation all the way down to $i = 0$ results in

$$\|\alpha^{k+1}_i - \alpha^*_i\| = \|\alpha^*_i\| \left(1 - \frac{\sigma^2_i}{\sigma^2_1}\right)^k.$$  \hfill (2.27)

Recall that $\sigma_i$ decreases with increasing $i$. Therefore $(1 - \sigma^2_i / \sigma^2_1) \leq 1$ for all $i$. This shows that the rate of convergence of the components associated with larger $i$ is always slower than of the first components. As a result, by choosing adequately an end $k$, the problematic components can be suppressed (cf. eq. (2.22)).

Algorithm 1 has two additional properties with respect to algorithm 2. The solutions are confined to the positive orthant in $\mathbb{R}^n$ by successive projections onto this orthant at every iteration. Secondly the rows included in each iteration are restricted according to eq. (2.4). These two operations can be incorporated into algorithm 2 by using diagonal matrices. However, these matrices change with each iteration and therefore so does the SVD. Providing a proof of convergence is beyond the scope of this work. Suffices to say that we carefully monitored the convergence of algorithm 1 for different numbers of diffusers and optical properties (fig. 2.3). In accordance with eq. (2.27), we observed near monotonic decrease of the cost as a function of iterations.
Chapter 3

Characterization of the fluence rate distributions that can arise from tailored cylindrical diffusers

Chapter summary

This chapter validates the light dosimetry model used in the previous chapter and studies the ability of tailored diffusers to shape the light dose distribution. Monte Carlo simulations and tissue phantom experiments are used to validate the model of light propagation in tissue used in this thesis. Once verified, I use the model to study the properties of the light distributions that arise from tailored cylindrical diffusers. An analytical expression for the loss of spatial modulation of the fluence rate contour lines is derived and compared to numerical solutions. The modulation transfer function is then used to investigate the attainable light dose distributions.

It is found that propagation of light through tissue rapidly degrades the spatial modulation of the fluence rate. Importantly, the modulation transfer function does not depend strongly on the optical properties.

The strong loss of modulation largely explains why, in the previous chapter, tailored diffusers only offered a marginal improvement of the light dose distribution over conventional ones. Furthermore, the robustness of this transfer function to changes in the optical properties also explains the observations from the previous chapter that, upon varying the optical properties, the original treatment plan could be largely recovered by dose re-normalization.
Chapter 3. Characterization of the forward problem

This chapter has been adapted from two previously published works:


[Rendon et al. 2007]: doi:10.1117/12.700996 in Proceedings of the SPIE

3.1 Introduction

The inverse problem of PDT treatment planning was addressed in Chapter 2. The main conclusion of that chapter was that using tailored cylindrical diffusers did not represent a real improvement over using conventional ones for prostate therapy. This result was somewhat unexpected given the added freedom offered by tailored diffusers to shape the light dose distribution. This chapter examines the light dose distribution arising from a tailored diffusers in order to explain the lack of improvement observed.

The chapter adopts a different structure than other chapters in this thesis, reflecting its largely theoretical nature. Section 3.2 goes into detail about the choice of the point source function that was used in the previous chapter (eq. (2.1)). It also present some numerical and experimental validation of the function chosen. Next, in section 3.3, we present a formalism used to characterize the shape of the light dose distribution and how it looses modulation as it propagates through the tissue. The chapter finishes by discussing the implications for PDT treatment planning and conformal light delivery.

3.2 Light propagation model

Recall that the fluence rate (in this chapter we revert to using fluence rate rather than light dose) arising from a cylindrical diffuser can be expressed as the convolution of the diffuser’s longitudinal emission profile with a suitable point source function (eq. (2.2)). The point source function, or kernel, describes the fluence rate for a unit power point source in a homogeneous medium characterized by a set of optical properties. Expressing the fluence rate as a convolution assumes that the principle of superposition holds, that
the tissue is homogeneous (i.e. that $G(r)$ is shift invariant), and that all source elements emit with the same angular distribution. It, otherwise, does not require isotropic emission. This is particularly useful when studying diffusers that emit with strong polar anisotropy in low albedo regimes [Menon et al. 2005].

When $S(z')$ describes an infinitesimally thin cylindrical diffuser of length $L$, placed along the $z$-axis, and cylindrical symmetry is used (assuming azimuthal symmetry of the kernel function), the fluence rate $\Phi(r)$ can be expressed as:

$$\Phi(\rho, z) = \int_{L} G(\rho, z - z') S(z') dz',$$

(3.1)

where $\rho = \sqrt{x^2 + y^2}$ is the distance perpendicular to the $z$-axis, and the integration is carried out over the length of the diffuser. An important consequence of expressing the fluence rate as in eq. (3.1) is that the eigenfunctions associated with this expression have the form $\exp(ikz)$ (strictly speaking, this is only true when $L \to \infty$). This means that the fluence rate distribution preserves the source’s spatial frequency. These eigenfunctions will be used in section 3.3 to examine the ability to conform to a given geometry.

3.2.1 Choices of kernel functions

The standard solution for an isotropic point source via the diffusion approximation has the form [Star 1997]:

$$G_{\text{diff}}(\rho, z - z') = \frac{\mu_{\text{eff}}^2}{4\pi\mu_a} \exp\left( -\frac{\mu_{\text{eff}} \cdot r}{r} \right),$$

(3.2)

where $r^2 = (\rho^2 + (z - z')^2)$. The effective scattering coefficient $\mu_{\text{eff}}^2 = 3\mu_a(\mu_a + \mu_s')$ is defined in terms of $\mu_a$ and $\mu_s' = \mu_s(1 - g)$, the absorption and reduced scattering coefficients respectively. The quantity $g$ is the average cosine of the scattering phase function, also called scattering anisotropy. This was the form used in Chapter 2 and derived in subsec. 1.3.2.

The main limitation of eq. (3.2) is that it fails to accurately describe the fluence rate in the proximity of the source. A useful heuristic solution to this problem is to add a
Figure 3.1 – Comparison between $G_{\text{diff}}$ (---) and $G_{\text{heu}}$ (−−) for various optical properties.

For reference, point source functions found via Monte Carlo simulations are also shown for two scattering anisotropies: $g = 0.0$ (+) and $g = 0.9$ (●). The optical properties correspond to $\mu_s' = 10$ cm$^{-1}$ and $\mu_a = 0.1$, 0.5, and 1 cm$^{-1}$. These resulted in effective attenuation coefficients, $\mu_{\text{eff}} = 1.73$, 3.89, 5.53 cm$^{-1}$.

collimated term and normalize to ensure energy conservation[Griff & Rinzema 2001]. This approach yields:

$$G_{\text{heu}}(\rho, z - z') = \frac{1}{4\pi} \left( \frac{a' \mu_{\text{eff}}^2 \exp(-\mu_{\text{eff}} \cdot r)}{\mu_a r} + \frac{\exp(-\mu' \cdot r)}{r^2} \right), \tag{3.3}$$

where $\mu'_t = \mu_a + \mu'_s$ and $a' = \mu'_s/\mu'_t$. Normalization leads to the extra $a'$ factor (c.f. eq. (3.2)). The second term in the RHS is the collimated component that represents the loss of primary photons due to absorption or scattering, together with the geometric dilution of the photon field. This term closely resembles the primary fluence rate introduced in section 1.3, with the substitution of $\mu_s$ for $\mu_s'$.  

Griff & Rinzema [2001] also suggest replacing $\mu_{\text{eff}}$ by $\mu_{\text{eff}}'$, the positive root of

$$\tanh^{-1}(\mu_{\text{eff}}'/\mu_t') = \mu_{\text{eff}}'/(\mu_t' \cdot a'),$$
For isotropic scattering (i.e. $g = 0$) this expression yields the exact effective attenuation coefficient derived from transport theory. Other choices of $\mu_{\text{eff}}$ have also been addressed in the literature (see for example [Graaff & Ten Bosch 2000, Pierrat et al. 2006] and references therein).

Figure 3.1 shows the difference between these kernels. For reference, Monte Carlo simulations\(^1\) for an isotropic source are included for two different scattering anisotropies. Notice the close similarity between the simulation with $g = 0$ and $G_{\text{heu}}$.

### 3.2.2 Comparison between modelled and simulated data for tailored diffusers

Let us see how these different point sources compare when used to calculate the fluence rate arising from a tailored diffuser. Assuming a diffuser 12 cm in length emitting with a sinusoidal profile of period 2 cm (i.e. $0.5(1 + \cos(\pi z))$), fig. 3.2 compares the resulting iso-dose contour lines for Monte Carlo simulated data ($g = 0$), and for fluence rate distributions calculated using $G_{\text{diff}}$ and $G_{\text{heu}}$. As expected, the approximation close to the origin improves as $\mu'_t$ increases (5.1 to 10.5 cm\(^{-1}\) from panel A to B). An increasing $\mu'_t$ shrinks the regime where the collimated component in eq. (3.3) dominates, thereby improving the behaviour close to the source. For extended sources such as cylindrical diffusers, using $G_{\text{diff}}$ seems to suffice, because, at any observation point $r$, the many point sources contributing to the fluence rate at $r$ are far enough such that $G_{\text{diff}}$ does not differ from more rigorous solutions.

An additional approximation comes from discretizing eq. (3.1) to compute $\Phi(r)$. The relative error in the fluence rate at a given $\rho$ due to sampling the source at a resolution $\Delta z'$, can be estimated to be of the order $\mathcal{F}_{2\pi/\Delta z'}/\mathcal{F}_0$. Here, $\mathcal{F}_k$ is the Fourier transform of

---

\(^1\)Monte Carlo simulations were carried out using the MCML code originally described in [Wang et al. 1995] and obtained from [http://omlc.org/software/mc/](http://omlc.org/software/mc/). This code uses the Heney-Greenstein scattering phase function which only depends on $g$. 

the kernel function in $k$ space, where $2\pi/\Delta z'$ is the spatial frequency associated with the sampling resolution. This result will become evident in subsec. 3.3.1 when the fluence rate from a sinusoidal line source is derived.

Figure 3.2 – Comparison between the fluence rate contour lines generated by: Monte Carlo (···), $G_{\text{diff}}$ (—), and $G_{\text{heu}}$ (−·−). The source profile is $0.5(1 + \cos(\pi z))$ extending from -6 to 6 cm. Only the central portion from -2 to 2 cm is shown. Note that no difference between $G_{\text{heu}}$ and the simulated data can be appreciated. The fluence rates for the contour lines of each figure were chosen to be equally spaced and have the same $\rho$ at $z = 0.5$ cm between both figures. (A) $\mu_a = 0.1$ cm$^{-1}$ and $\mu'_s = 5$ cm$^{-1}$; (B) $\mu_a = 0.5$ cm$^{-1}$ and $\mu'_s = 10$ cm$^{-1}$. These values correspond to $\mu_{\text{eff}} = 1.23$ cm$^{-1}$ and $\mu_{\text{eff}} = 3.89$ cm$^{-1}$ respectively.

There is another practical consideration for using $G_{\text{diff}}$ in treatment planning. While measuring $\mu_{\text{eff}}$ in vivo is relatively easy, measuring $\mu'_s$ and $\mu_a$ requires very careful calibration and positioning of the detectors. If one is prepared to use relative units of fluence rate for treatment planning, $\mu_{\text{eff}}$ is sufficient for determining the geometry of the light dose distribution. During actual light delivery, on-line dose monitoring can be used to ensure delivering the stipulated dose.
In summary, while there are better approximations, $G_{\text{diff}}$ is a suitable kernel function as long as it is not used in close proximity to the source (say \( \lesssim 2 \text{ mm} \), the exact number depending on $\mu'_t$). In fact, it may very well be the only kernel with parameters that can be actually measured in the clinical setting. Given these consideration, $G_{\text{diff}}$ will be used for the remainder of this chapter.

### 3.2.3 Comparison with experimentally determined fluence rate distributions

It remains to be answered whether $G_{\text{diff}}$ can indeed replicate experimental data. To determine this, the fluence rate distribution arising from a tailored cylindrical diffuser was measured and the results fitted to the fluence rate calculated using $G_{\text{diff}}$. The fitting should not only recover the model parameters, but it should also reproduce the shape of the fluence rate distribution. To simulate tissue, liquid phantoms were prepared in water from stock solutions of 20% Intralipid (Baxter) to provide scattering, and 2.85 mM Naphthol Green B (NGB, Cat. # N7257; Sigma) as an absorber.

**Experimental set-up**

A 5 cm tailored diffuser with a sinusoidal emission profile of period 1.7 cm was kindly provided by Walsh Medical Devices. The power emission profile, supplied by the manufacturer, was measured in air, at an angle perpendicular to the diffuser, with a small viewing angle photodetector and an accuracy better than 1 % (setup described in [Vesselov et al. 2004]). Two litre volumes of liquid phantoms were prepared with different sets of optical properties according to the formulations shown in table 3.1. The optical properties were chosen to reflect those found for PDT in the clinic. All experiments were carried out in a black plastic cubic tank 13 cm at the sides. Fluence rate maps were measured using a scattering bulb 0.85 mm in diameter (Cat. # IP85; Medlight) connected through an optic fibre to a photodiode. The scattering bulb was raster scanned...
in the horizontal plane using top mounted $x$ and $y$ micro-positioning stages (Cat. # NRT150/M; Thor Labs) at a 1 mm scanning resolution under computer control. The accuracy of the fluence rate measurements was better than 5%. A 635 nm diode laser was used for all measurements.

### Table 3.1 – Tissue phantom formulations

<table>
<thead>
<tr>
<th>Phantom</th>
<th>NGB [µM] ($\mu_a$ [cm$^{-1}$])</th>
<th>Intralipid [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>10.7 (0.17)</td>
<td>0.4</td>
</tr>
<tr>
<td>b</td>
<td>10.7 (0.17)</td>
<td>1.0</td>
</tr>
<tr>
<td>c</td>
<td>12.8 (0.20)</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Three different sources were measured: a point source, a diffuser in a catheter, and the bare diffuser. The experimental setup is shown in Fig. 3.3A. Only one side of the fluence rate map on the horizontal plane at the same height of the diffuser or point source was scanned. To determine $\mu_{\text{eff}}$, an identical scattering bulb, now used as a source, was placed at the centre of the tank and the fluence rate map on the horizontal plane was measured for the different phantoms Fig. 3.3B. The scattering bulb was then replaced with a 2 mm outer diameter clear plastic catheter where the diffuser was inserted. In a third set of measurements, the diffuser was placed in the tank without a catheter to examine the effects of the catheter Fig. 3.3C.

### Data fitting

Firstly, the fluence rate measurements needed to be aligned with respect to the diffuser’s emission profile. The alignment was achieved by finding the position of the peak of the cross-correlation function between the emission profile and the fluence rate along $x$, at the $y$ closest to the diffuser. For the point source, the same fluence rate sample was fitted to a spline and the position of the maximum determined. This procedure aligned the
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Figure 3.3 – Experimental setup for fluence rate measurements. (A) Collection geometry for diffuser measurements. Fluence rate contour map are shown for samples of the data acquired for the point source measurements (B), and for the diffuser measurements (C). The logarithm of the fluence rate is used.

data along \( x \). The \( z \) coordinate was taken to be the same for the source and the fluence rate map. Alignment along \( y \) is more difficult. This was done manually (keeping the same distance for each source) by adjusting \( y_0 \) in eq. (3.4). The adjustment was done until a good visual fit between the shapes of the predicted and measured fluence rate distributions was achieved.

After normalizing the data by dividing by the largest fluence rate, the results were fitted by minimizing the following cost: \( \sum_i \left\| \log(\phi_i) - \log(\sum_j G^{ij} s_j) \right\|^2 \), with

\[
G = K \frac{\exp(-\mu_{\text{eff}} (x^2 + (y - y_0)^2)^{1/2})}{(x^2 + (y - y_0)^2)^{1/2}}. \tag{3.4}
\]

Recall that \( s_j \) is the source’s emission profile. \( G \) is just a simplified version of \( G_{\text{diff}} \) with only two parameters to fit: \( K \) and \( \mu_{\text{eff}} \). The parameter \( K \) should be directly proportional to the total optical power and to \( \mu_s \) (c.f. eq. (3.2) with \( \mu_a \ll \mu_s \)). The minimization was carried out using the constrained nonlinear minimization function \texttt{lsqnonlin()} in MATLAB’s Optimization toolbox.
Fit results

The fitting of the data was very satisfactory. Not only was the shape of the fluence rate maps well predicted (fig. 3.4), but also, the recovered attenuation coefficients were consistent between the three sources (table 3.2). Finally, $K$ correlated well with the Intralipid concentration (fig. 3.5 B). In fact, for the combined data, the Pearson’s correlation coefficient was 0.98 ($p=7 \cdot 10^{-7}$). Additionally, it was interesting to find that the presence of a clear catheter did not affect the predictions of the model. Since diffusers are always placed within catheters in the clinical setting, this result is of great practical value. However, a note of caution about the choice of $y_0$ is necessary. Figure 3.5 A shows that while $\mu_{\text{eff}}$ does not change much ($\pm 5\%$) over a range of 3 mm for $y_0$, the value of $K$ does ($\pm 100\%$).

In conclusion, this section has provided evidence from Monte Carlo simulations and experimental measurements confirming the choice of $G_{\text{diff}}$, in combination with eq. (3.1), as a model for the fluence rate arising from tailored diffusers.

### Table 3.2 – Recovered $\mu_{\text{eff}}$ for the different phantoms.

<table>
<thead>
<tr>
<th>Source</th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point source</td>
<td>1.6</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Diffuser in catheter</td>
<td>2.5</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Bare Diffuser</td>
<td>3.2</td>
<td>3.0</td>
<td>3.1</td>
</tr>
</tbody>
</table>

All $\mu_{\text{eff}}$ in cm$^{-1}$

### 3.3 Light dose distributions

In the two dimensional single diffuser case presented here, the concept of conformal light delivery is equivalent to matching an iso-dose line to a predefined boundary line. A target object (or a feature part of a larger object) to which the light dose distribution is to be
Figure 3.4 – Results of the fit to the fluence rate maps for the different phantoms (a-c) and sources: (A) point source, (B) diffuser placed in a catheter, and (C) bare diffuser. The logarithm of the fluence rate for different $y$ spaced every 3 mm is shown. The measured results are indicated by the (+) symbols and the modelled data by the solid lines.
Figure 3.5 – The value of \( \mu_s \) strongly depends on the choice of \( y_0 \), although consistent solutions across all phantoms can be found. (A) While the value of \( \mu_{\text{eff}} \) changes very little as a function of \( y_0 \), the value of \( K \) and hence of \( \mu_s \) is very sensitive to the particular choice of \( y_0 \). (B) Choosing \( y_0 \) in order to obtain a good visual fit of the contour lines leads to good correlation between \( K \) and the Intralipid concentration. The \( K \) for the different sources was normalized to their sum per source. The three sources are: point source (+), catheter in diffuser (○), and bare catheter (×). The line of best fit for the complete data set is also shown.

matched against can be described in terms of three distances: \( \rho_0 \) the distance of the object’s centre to the diffuser, \( w \) half its width measured along \( z \) (spatial frequency), and \( d \) half its depth measured along \( \rho \) (amplitude). Figure 3.6 depicts a sinusoidal iso-dose line matching an ellipsoid, representing a minimal object that is fully characterized by its semi-axes, \( w \) and \( d \). In this depiction the object is to be avoided (light dose matched to the bottom side). Shifting the phase by half the period would represent conforming to an object included in the irradiation field (light dose matched to the top side).

This section pertains to the derivation of an expression that relates \( d \) and \( \rho_0 \) for spatial frequencies given by \( k = \pi/2w \). This relationship characterizes the interplay
Chapter 3. Characterization of the forward problem

Figure 3.6 – Depiction of the geometrical parameters. Panel (A) represents a sinusoidal iso-dose line of amplitude $d$, matching an elliptic object centred at $\rho_0$. The diffuser is represented by the thick line at the bottom. The spatial frequency of the object corresponds to $k = \pi/2w$. Here, the act of avoiding a critical object is depicted. Panel (B) shows the same parameters for two contour lines for the emission profile $S = 0.5 + 0.5 \cos(\pi z)$. The two lines correspond to two different regimes: (a) type contour lines are fully detached from the diffuser, while (b) contour lines are continuous with the $z$ axis (diffuser).

between these geometrical parameters and therefore specifies the attainable light dose distributions.

3.3.1 Iso-dose lines for a sinusoidal source

Consider the source profile $S(z') = (1 + M \sin(kz'))/2$ defined over all $z'$. An example is shown in fig. 3.6B. The constant term is added to ensure positive fluence rates, and the modulation $M$ is a number between 0 and 1, where 0 corresponds to a flat line. Choosing this source function has two advantages. First, any arbitrary source profile can be described by a sum of such terms via a Fourier series. Second, an analytical solution for the fluence rate exists. Since harmonic functions are eigenvalues associated with eq. (3.1), the problem of defining the attainable fluence rate distributions can be
translated to understanding the loss of source modulation as a function of spatial frequency. Additionally, the steep decay of the kernel function makes this source, otherwise of infinite length, a good approximation to a finite diffuser emitting with a sinusoidal profile.

Replacing $S(z')$ in eq. (3.1), making the substitution $u = z - z'$, and using the parity of $G$ yields:

$$\phi(\rho, z) = \int_0^\infty du G(\rho, u) + M \sin kz \int_0^\infty du G(\rho, u) \cos ku.$$

(3.5)

The second integral in the RHS is the Fourier cosine transform of the Green’s function. The first integral corresponds to the Fourier cosine transform evaluated in the limit of $k \to 0$. The Fourier cosine transform of $\exp(-\mu_{\text{eff}} \rho) / \rho - 1$ is $K_0(\rho(\mu_{\text{eff}}^2 + k^2)^{1/2})$, where $K_n$ is the $n$-th order modified Bessel function [Oberhettinger 1990]. Denoting the Fourier cosine transform as $\mathcal{F}(\rho, k)$ and rearranging, yields for the fluence rate:

$$\phi(\rho, z) = \mathcal{F}(\rho, 0) + M \sin(kz) \mathcal{F}(\rho, k).$$

(3.6)

With this expression, the modulation transfer function for the fluence rate becomes

$$M(\rho) = \mathcal{F}(\rho, k) \mathcal{F}(\rho, 0)^{-1} \cdot M(\rho = 0).$$

This function decreases monotonically for increasing $\rho$ and $k$, and increases with $\mu_{\text{eff}}$. The modulation transfer function was used in subsec. 3.2.2 to estimate the relative error due to discretizing with spatial frequency $k = 2\pi / \Delta z'$. In this case, the remaining modulation due to sampling is the relative error in the fluence rate.

Equation (3.5) does not lend itself to a straightforward geometrical interpretation. The geometrical information is contained in the iso-dose contour lines. A first order Taylor expansion in $\rho$ of $\phi(\rho, z)$, around $\rho_{\text{DC}}$, is used to approximate the contour lines. The subscript $\text{DC}$ is used to emphasize that this value is selected at the $z$ where the sinusoidal component vanishes, for example at $z = 0$ for the $S(z')$ defined above. Solving for $\rho$ yields:

$$\rho = \rho_{\text{DC}} + \phi - \mathcal{F}_0(\rho_{\text{DC}}) - M \sin(kz) \mathcal{F}_k(\rho_{\text{DC}}) \overline{\mathcal{F}_0'(\rho_{\text{DC}}) + M \sin(kz) \mathcal{F}_k'(\rho_{\text{DC}})},$$

(3.7)
where \( k \) has been placed as a subscript. The primed functions denote differentiation with respect to \( \rho \). By definition of \( \rho_{DC} \), the quantity \( \phi - F_0 \) vanishes for all \( \rho \) belonging to the contour level of fluence rate \( \phi_0 = \phi(\rho_{DC}, z = 0) \). Therefore, the iso-dose lines for eq. (3.5) can be approximated by:

\[
\rho = \rho_{DC} - \frac{M \sin(kz)F_k}{F'_0 + M \sin(kz)F''_k}.
\] (3.8)

Note that eq. (3.8) takes negative values for some \( z \). Since \( \rho \) is a radial distance such values are not allowed. They correspond to positions along \( z \) where the diffuser emits with less power than that corresponding to \( \phi_0 \), and are thus set to zero. This condition is identified in fig. 3.6B by the contour line labelled (b). The regime where \( \rho > 0 \) for all \( z \) is indicated in the same figure by the contour line labelled (a). For \( G_{diff} \), the transition between these two regimes occurs at \( \phi = 3\mu^2_{eff}/(4\pi\mu_{a})\log(1 + k^2/\mu^2_{eff}) \).

Equation (3.8) has the property that it is only stated in geometrical terms and reference to the fluence rate is hidden in \( \rho_{DC} \). Furthermore, by using \( G_{diff} \), all distances become multiplied by \( \mu_{eff} \), hence becoming dimensionless parameters.

The second order term in the Taylor expansion leading to eq. (3.7) has the form \( \mu^2_{eff} \cdot f(\rho_{DC})(\rho - \rho_{DC})^2 \), where \( f(\rho_{DC}) \) is a steeply decreasing function of \( \rho_{DC} \). Therefore eq. (3.8) is only valid for for small \( \mu_{eff} \) and far from the diffuser.

### 3.3.2 Attainable light dose distributions

Equation (3.8) can be used to calculate the relationship between the parameters identified in fig. 3.6, and hence characterizes the shapes of contour lines that can be attained. The amplitude \( d \) and distance \( \rho_0 \) are given by \( (\max(\rho) - \min(\rho))/2 \) and \( (\max(\rho) + \min(\rho))/2 \) respectively. Since the maxima and minima of \( \rho \) occur for the same values of \( z \) where the source profile takes its maxima and minima, these values are easy to compute. When
Figure 3.7 – Maximum $d$ attainable for a given spatial frequency $k$ (in cm$^{-1}$) and distance away from the diffuser $\rho_0$. The analytical (− −) and numerical (—) solutions to the contour lines of eq. (3.6) are shown. Notice that the discrepancy between the two increases with larger $\mu_{\text{eff}}$ and smaller $k$. The source modulation, $M$, has been set to its maximum value of one.

For $\rho_{\text{DC}}$ in regime (a), which satisfies the implicit inequality $\rho_{\text{DC}} < -M F_{k}/(F'_0 - M F'_k)$, eq. (3.9) and eq. (3.10) take the combined form

$$d = \rho_0 = \rho_{\text{DC}} - \frac{M F_k}{F'_0 - M F'_k}.$$  

Figure 3.7 shows these relationships. To evaluate the effects of the first order approximation, the exact numerical solutions to the contour lines of eq. (3.6) are also shown. The initial straight line corresponds to regime (a), which represents the diffuser crossing the target surface. For larger $\rho_0$, the values of $d$ decrease as the initial intensity and modulation of the source are lost through absorption and scattering. Furthermore, the
maximum $d$ decreases as the width of the object becomes smaller. Notice also that the analytical solution is independent of $\mu_{\text{eff}}$. This is not the case for the numerical solutions. However, the analytical solution does approximate the numerical one for small $\mu_{\text{eff}}$ and large $k$.

In summary, if the light dose is to be conformed to a structure of width $w$ and depth $d$, equations (3.9)–(3.11) determine the maximum $\rho_0$ that can lead to a conformal dose.

Finally, the fact that the function $d(\rho_0)$ is independent of $\mu_{\text{eff}}$ implies that the shape of the light dose distribution is large insensitive to changes in $\mu_{\text{eff}}$. This was verified numerically for the exact solution of eq. (3.6) and is shown in fig. 3.8. Notice that regime (a) is particularly robust to changes in $\mu_{\text{eff}}$. For regime (b), the robustness decreases as the object becomes wider (decreasing $k$).

### 3.4 Discussion

This chapter provided a geometrical description of the attainable fluence rate distributions for a single diffuser with a customizable emission profile. It offered an explanation to the observation of Chapter 2, where tailored diffusers do not show a significant improvement to conformal light delivery over conventional ones. The explanation takes the form of the transfer function for the spatial modulation of light propagating in tissue (equations (3.10) and (3.11)). This transfer function not only peaks close to the diffuser, but also decreases steeply as the distance away from the diffuser increases (fig. 3.7).

Expressing the fluence rate as the convolution of the source with a point source kernel function is a common approach to these types of problems [Arnfield et al. 1989]. Typically, a point source function obtained via diffusion theory has been assumed [Arnfield et al. 1989, Altschuler et al. 2005]. However, this kernel in principle suffers from at least two flaws. It does not account for highly anisotropic scattering and it fails to account for the fluence rate in close proximity to the source. The Monte Carlo simulations in fig. 3.1
Figure 3.8 – Effects in $d$ due to changes in the optical properties. Three different spatial frequencies of the emission profile are presented as labelled above each panel. Each panel shows the ratio of $d$ to its maximum value for each spatial frequency depicted. The maximum values of $d$ for each panel are, from left to right, 0.23, 0.46, and 0.68 cm.

with $g = 0.9$ and $g = 0$ (isotropic scattering) indicate that scattering anisotropy has no effect for the ranges of optical properties seen in the clinical practise. Furthermore, for extended sources such as cylindrical diffusers, $G_{\text{diff}}$ provides a good approximation beyond at least 2 mm (fig. 3.2). This distance is of the same order as the size of the diffusers and the fluence rate probes, and hence, sufficient.

Cylindrical diffusers do not radiate in air like a collection of isotropically emitting point sources. For the diffusers used in this study, Vesselov et al. [2005] found that the emission is strongly forward directed, peaked at approximately 30°. Monte Carlo studies by us have previously revealed that in the interstitial setting, such emission anisotropies were of little consequence [Rendon et al. 2005]. This, however, had not been experimen-
tally validated. Murrer et al. [1996] had previously measured the resulting fluence rate distribution of conventional cylindrical diffusers in a single intralipid concentration with no added absorber (0.3 % Intralipid), and found good agreement with the experimental measurements. We have measured the fluence rate distribution arising from a tailored diffuser with a sinusoidal emission profile covering a range of $\mu_{\text{eff}}$ between 1.6 and 3.2 cm$^{-1}$. Our results for the tailored diffusers agree with those of Murrer et al. [1996] for all optical properties tested. It is remarkable that measurements of the emission profile in air at 90° yielded the accuracy observed in our data set. This is very encouraging if this diffusers are to be used in the clinic. Additionally, we found that placing the diffuser in a clear catheter did not affect the fluence rate distribution. Overall, these results indicate that the prediction of the light dose under this formalism is quite satisfactory, and validate the choices made in Chapter 2 for the solution of the inverse problem.

Equation (3.6), which expresses the fluence rate for a sinusoidal emission profile, offers additional insight about the ill-posedness of the inverse problem already touched upon in Chapter 2. It says that source components with a spatial frequency $k$ are filtered approximately as $\exp(-\rho((\mu_{\text{eff}}^2 + k^2)^{1/2})/\rho(\mu_{\text{eff}}^2 + k^2)^{1/2})$, which is a very steep function of $k$ that asymptotically approaches zero. For the inverse problem it says that noise of high spatial frequencies is amplified by the inversion procedure when the target fluence rate is specified far from the diffuser (or for large $\mu_{\text{eff}}$).

The interplay between the different geometrical parameters found in subsec. 3.3.2 can be placed in context as follows. For a $\mu_{\text{eff}}$ of 3 cm$^{-1}$ and a surface feature of 0.8 cm by 2 cm (2$d$ by 2$w$ respectively), the maximum $\rho_0$ is approximately 0.6 cm. This figure hints that the potential for conformal light delivery is limited to lesions with small spatial frequency, where the diffuser can be placed close to the target surface features. This explains the little improvement brought by using tailored diffusers in Chapter 2.

It was also observed in Chapter 2 that homogeneous changes in optical properties affected little the shape of the light dose distribution. To a first approximation, the
function $d(\rho_0)$ also depends only very slightly on the optical properties (fig. 3.7). Solving numerically does show a dependence on the optical properties (fig. 3.8). However, the dependence is not very strong. In fact, for contour lines in regime (a), changing $\mu_{\text{eff}}$ has little effect. These results provide an explanation of the observations in Chapter 2 sec. 2.3.3 that re-normalizing the dose after changing the optical properties restored the prostate coverage with no added over-dosing of the rectum.
Chapter 4

Exploration of the potential use of erythropoietin as an adjuvant to PDT of brain tumours

Chapter summary

The use of Photodynamic therapy (PDT) as an adjuvant to surgical resection for the treatment of malignant gliomas has been studied for over two decades with mixed success. While, in principle, the preferential uptake by the tumour of the photosensitizer should result in selective tumour treatment, the inherent sensitivity of normal brain degrades this selectivity. Here, I study the potential of using erythropoietin (EPO) as a neuro-protectant to decrease the sensitivity of normal brain and rescue the treatment selectivity, thereby increasing the therapeutic index. Using primary neuronal cultures, I have evaluated EPO’s ability to protect neurons from aminolevulinic acid (ALA) mediated PDT. Complementing these \textit{in vitro} studies, EPO’s ability to protect normal rat brain \textit{in vivo} from Photofrin mediated PDT was also tested.

The results largely support that EPO is unable to protect cultured neurons and normal brain from PDT mediated cell kill, under the conditions tested.

Various explanations are given for this lack of an effect. I also outline scenarios under which EPO or other neuro-protectants can successfully improve the therapeutic index, in particular, for the treatment of age-related macular degeneration using PDT.
4.1 Introduction

Successful treatment of malignant gliomas remains elusive, with less than 3% of patients surviving more than five years [Ohgaki & Kleihues 2005]. Tumour invasiveness constitutes one of the main hurdles for improving treatment outcome [Preusser et al. 2006]. In fact, more aggressive tumour resection is clearly associated with longer survival [Lacroix et al. 2001]. However, improving survival without concomitantly increasing side-effects requires a treatment with a high tumour selectivity. PDT has been investigated for over two decades as an adjuvant to surgery as a means to selectively clear remaining tumour close to the resection margin [Styli & Kaye 2006a].

Multiple studies have highlighted the preferential accumulation of photosensitizers within brain tumours [Lilge & Wilson 1998]. However, this selectivity is not preserved after light delivery due to the intrinsically higher sensitivity to PDT of normal brain, in comparison to tumorous tissue [Lilge et al. 1996]. Selectivity can be restored by two different means. A common approach has sought to increase PDT’s tumour selectivity by using more selective photosensitizers [Reuther et al. 2001, Jori 1996] or dose delivery regimes [Bisland et al. 2004]. Alternatively, the sensitivity to PDT of normal tissue can be reduced. We pursue here the latter approach using erythropoietin (EPO) as a neuroprotectant.

EPO is a well-known cytokine primarily responsible for erythropoiesis. Recently, additional roles in development and tissue protection have been ascribed to it [Li et al. 2004, Brines & Cerami 2005]. Physiologically, EPO and its receptor (EPOR) are tissue protective primarily by inhibiting apoptosis via autocrine and paracrine signalling [Sirén et al. 2001]. Exogenous EPO has been shown to cross the intact blood brain barrier [Banks et al. 2004, Brines et al. 2000] and confer protection to neurons [Digicaylioglu & Lipton 2001] and vascular endothelial cells [Chong et al. 2002]. Tissue culture studies have highlighted EPO mediated protection from glutamate [Morishita et al. 1997], hypoxia [Sinor & Greenberg 2000], and NO stress [Digicaylioglu & Lipton 2001], among other
insults. \textit{In vivo}, EPO protects from a variety of stressors which include ischemia [Leist \textit{et al.} 2004], blunt trauma [Brines \textit{et al.} 2000], and radio-surgically induced brain injury [Erbayraktar \textit{et al.} 2006]. The only clinical trial reported that has tested EPO to reduce stroke morbidity, showed promising improvements in neurological outcome and lesion size reduction versus saline only [Ehrenreich \textit{et al.} 2002]. Other trials are underway.

Several pathways have been proposed to work in concert leading to EPO mediated neuroprotection (fig. 4.1). In analogy with the classical erythroid pathway, binding of EPO by EPOR changes the receptor conformation, thereby phosphorilating JAK2 (Janus tyrosine kinase 2). Alternative binding to a heterodimer formed by EPOR and the $\beta$ common receptor ($\beta$CR – a receptor subunit shared by interleukin 3 receptor and others) has also been described [Brines \textit{et al.} 2004], but remains controversial [Um \textit{et al.} 2007]. Several downstream anti-apoptotic pathways have been found to be triggered by JAK2. These include the activation of Akt [Chong \textit{et al.} 2003b] and MAPK [Kilic \textit{et al.} 2005a]; up-regulation of BCL$_{\text{XL}}$ [Wen \textit{et al.} 2002] via STAT5 [Um & Lodish 2006]; and translocation of NF$\kappa$B to the nucleus [Digicaylioglu & Lipton 2001] increasing the ability to cope with oxidative stress via transcriptional activation of various superoxide dismutases and members of the glutathione antioxidant system [O’Neill & Kaltschmidt 1997]. Consistent with these mechanisms, EPO has been shown to only protect from apoptosis.

The proposed strategy is only effective if tissue protection is preferentially conferred to normal tissue versus tumour. After years of using EPO in oncology to alleviate chemotherapy induced anaemia, several recent trials have been terminated due to increase mortality in EPO treated groups, often due to vascular thrombosis [Henke \textit{et al.} 2003, Leyland-Jones \textit{et al.} 2003, Wright \textit{et al.} 2007]. In contrast, other studies seem to suggest that EPO improves treatment efficacy [Larsson \textit{et al.} 2004], likely due to improved oxygenation. Presently, the impact of EPO as a growth factor on tumour growth is under active investigation [Hardee \textit{et al.} 2006, LaMontagne \textit{et al.} 2006]. Furthermore, deriva-
Figure 4.1 – Proposed signalling pathways involved in EPO mediated tissue protection. Binding of EPO to tissue-protective erythropoietin receptor (EPOR–βCR) activates JAK2, which then engages secondary signalling pathways involving MAPK, Akt, NF-κB) nuclear translocation, and BCL\textsubscript{xL} up-regulation via STAT5. Ultimately, these secondary signalling events lead to the activation of several anti-apoptotic events which include caspase inhibition, BCL\textsubscript{xL} up-regulation, and GSK-3β, which inhibits mitochondrial permeability transition. Finally, EPO modulates the activity of calcium channels through phospholipase C (PLC), thereby reducing the release of excitatory neurotransmitters and increasing nitric oxide production.

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Brines M. and Cerami A. *Emerging biological roles for erythropoietin in the nervous system*, Nature Reviews Neuroscience 6, 484-494 (2005), DOI: 10.1038/nrm1687, Copyright (2005).
tives of EPO have been identified that are non-haematopoietic but still tissue protective [Leist et al. 2004, Doggrell 2004, Coleman et al. 2006], and that, therefore, decrease the thrombotic side-effects.

This study seeks to establish whether there is potential for using EPO to improve the therapeutic ratio of brain PDT. This was done by assessing EPO mediated protection from PDT in vitro and in vivo in normal tissue. Firstly, I tested whether EPO improves the survival of primary cortical neurons treated with ALA mediated PDT. Subsequently, I determined whether EPO pre-treatment protects normal rat brain from direct cell kill caused by Photofrin mediated PDT, as measured by changes in the volume of brain necrosis induced by PDT.

4.2 Materials and Methods

4.2.1 Animals

All animal usage protocols were approved by the Animal Care Committee at the University Health Network, in agreement with federal, provincial and institutional regulations. All efforts were made to minimize the number of animals used and their suffering. For preparing neuronal cultures, time-pregnant Lewis rats were used. For in vivo PDT, 200-220 g female Lewis rats were used. All animals were purchased from Charles Rivers Laboratories (Montreal, Canada). Animals had free access to water and food.

4.2.2 Cell culture

Primary cortical cultures

Pregnant rats were placed under 5% isoflurane anaesthesia and embryonic day 16-17 pups were removed via C-section. From there on, all resection procedures were done over a bed of ice. All solutions used were pre-gassed to balance CO$_2$ (placed over-night
in 5 %CO$_2$/air balanced incubator in a gas-permeable tissue culture flask). The brains were resected and placed in cold EBSS (Earls Balanced Salt Solution). Meninges were carefully removed by cutting across the midline, and peeling the meninges over each hemisphere, from the olfactory bulb back towards the dorso-caudal aspect of the cerebrum. The neo-cortex was carefully removed, avoiding the hippocampus (when present). The cortices were placed in 1:1 EBSS and culture medium (Neurobasal Medium Cat.# 21103-049; 1:50 B27 Supplement Cat.#17504-044 Lot #; 0.25 mM Glutamax Cat.# 35050-061;0.25 mM L-glutamine Cat.# 25030-149; 1:50 Penicillin-Streptomycin Cat.# 15140-148; Invitrogen Corp. ) until dissociation step (less than 2 hours). Cortices were dissociated with papain following the vendor’s instructions (Papain Dissociation System, Cat. # LK003150; Worthington Biochemical Corp. Lakewood, USA)Briefly, cortices were incubated in a papain-DNase solution (20 U/ml papain, 0.005% DNase in EBSS, respectively) for 30-45 min at 37°C gently dissociated using a cut-end 1000 µl pipette tip, strained through a 70 µm mesh (Cat. # 352350; BD Falcon), and centrifuged at 300g for 6 min. Cells were then layered over an albumin density gradient and centrifuged at 70g for 6 min to remove cellular fragments. The cells were subsequently resuspended in culture medium and plated at a density of 2 × 10$^5$ cells/cm$^2$ on poly-Ornithine (Cat.# P3655; Sigma, Canada) coated (10 µg/ml poly-Ornithine in water overnight, washed by triplicate, and dried by aspiration) multi-well plates (Costar 12 or 48 well plates Cat.# 3512,3548; Corning Life Sciences). For immunocytochemistry, cells were plated on 15 mm round glass coverslips (also coated). One half of the medium was replaced with fresh medium every 3-4 days.

**Glioma cell lines**

CNS-1 rat astrocytoma, 9L rat gliosarcoma and U87 human glioblastoma cell lines were passaged twice a week in medium (RPMI-1640, D-MEM, and alpha-MEM respectively) containing 10 % fetal bovine serum (Cat. # A15-701; Cansera, Canada).
4.2.3 Western blotting

Primary cortical neurons \((2 \times 10^7)\) were lysed in M-PER protein extraction reagent (Cat. # 78503; Pierce). Other tissues were homogenized with a mortar and pestle in a Tris buffer (50 mM Tris pH8, 5mM EDTA, 1mM PMSF, 1 per 10ml Complete protease inhibitor cocktail tablet Cat.#1836153; Roche Applied Sciences). When needed, DNA was sheared by ultrasonication on ice with less than five 1-2 sec pulses. Protein content was measured using Bradford’s method (Cat.# 500-0006; Bio-Rad). Equivalent amounts of protein \((30 \mu g)\) were boiled for 5 min in loading buffer containing 0.1M DTT, resolved on Tris-Glycine 10% SDS gels, and then electroblotted onto PVDF membranes (Cat.# IPVH 304 F0; Millipore) at 4°C overnight. After blocking with 5% milk powder (Cat.# SKI400.500; Bioshop, Canada) in TBS-T for 2 h, membranes were incubated with EPOR antibody (1:200 clone M-20 Cat.# sc697 Lot#B2207; Santa Cruz Biotechnology) for 2 hr RT, and washed 4x5 min in TBS-T. This was followed by incubation with horseradish peroxidase-conjugated secondary antibody (1:5000 Cat.#111-036-003; Jackson Immunoresearch Laboratories) for 1 hr RT. After another wash, detection was achieved using the ECL method (Amersham ECL Cat.# RPN2106; GE Healthcare Life Sciences).

4.2.4 Challenge and EPO incubation

Neurons were cultured for 10 days prior to glutamate challenge to allow full expression of the glutamate receptor [Craig et al. 1993]. For other challenges, neurons were used between 7-8 days in vitro. EPO (10 U/ml human recombinant erythropoietin, Eprex; Janssen-Ortho,Canada) was added 18 hr prior to challenge, while replacing the cultured medium with one half fresh medium and one half spent medium filtered sterile.

Glutamate challenge was performed by replacing the culture media with glutamate (Cat.# G2834, Sigma) in HEPES buffer (17mM HEPES, 20mM glucose, 1.2 mM NaCl,
3 mM NaHCO$_3$, 5mM KCl, 1.8 mM CaCl$_2$, 1 mM Na pyruvate, 0.01 mM glycine) for the indicated times and then returned to fresh culture medium.

Staurosporine (Cat.# S6942, Sigma) and camptothecin (Cat.# C9911, Sigma) were dissolved in DMSO (DMSO final concentration less than 0.5%) and added directly to the EPO containing medium for 24 hr, time after which viability was assayed.

5-aminolevulinic acid (ALA; Cat.# A7793, Sigma) was added to the EPO containing media 4 hr prior to light exposure, time at which medium was replaced with fresh culture medium. Light exposure was carried out using a bank of unfiltered halogen lamps at 22 mW/cm$^2$. Irradiance homogeneity better than ±10% was achieved by using a diffusing sheet used for photography (Lee Filters, UK). A water tank made in plexiglas 7 cm along the light path was used to filter the infrared and minimize heating. The temperature was monitored to be in the range 30-35 °C. After irradiation, cell were placed back in the incubator and viability was assayed 24 hr later.

PDT treatment of tumour cell lines with 200 μg/ml ALA or 2.5 μg/ml Photofrin (Axcan Pharma; Canada) was performed as follows. After 4 hr incubation with ALA (or 24 hr with Photofrin), the medium was replaced for full medium including serum and illuminated using the same bank of halogen lamps but filtered with a long pass 600 nm red cellulose sheet (Lee Filters, UK). The water tank was also used for these experiments. Viability was assayed 24 hours post light delivery.

4.2.5 Determination of cell viability and survival

Cell viability

A metabolic assay based on the oxidation of non-fluorescent resazurin (Alamar Blue) to fluorescent resorufin was employed. 20 μl of resazurin (Cat.# R7017; Sigma) stock solution were added to each well (4 μM final concentration). An hour later (experimentally determined to yield a good signal to noise ratio, while still showing a linear response)
resorufin fluorescence (560 ex. / 595 em.) was measured in a plate reader (SpectraMax 5M; Molecular Devices); multiple treatment conditions were read with the same gain settings and mean blank fluorescence (at least 6 wells per plate) was subtracted from each reading. Viability was calculated as the ratio of the fluorescence signals of treated versus control.

**Cell survival**

Cell survival was determined by fluorimetric reading of nuclear uptake of impermeable dye Sytox Orange pre- and post-permeabilization of the cell membrane. Sytox Orange undergoes an over 500 fold fluorescence enhancement upon binding to nucleic acids, therefore having very low background of the unbound dye. 400 µl of Sytox Orange (0.5 µM) in culture medium were added each well of a 48 well plate and incubated for 10 min RT. Fluorescence (540 ex. / 570 em.) was then read in a plate reader. Subsequently, 20 µl Triton-x (0.4% final concentration) were added to each well, followed by a 15 min incubation RT. This length of time was determined to be sufficient to allow full cell permeabilization. Fluorescence was read again under the same wavelengths and detector gains. For each well, cell survival was determined as $1 - \frac{\text{Sample}_{\text{pre}} - \text{Blank}_{\text{pre}}}{\text{Sample}_{\text{post}} - \text{Blank}_{\text{post}}}$, where the blanks were the average of 4 wells in the same plate that only contained culture medium. At least six wells were assigned to each condition and their average was used to account for one independent experiment.

**4.2.6 PDT of the rat brain**

20 hours prior to irradiation, animals received 6.25 mg/Kg of Photofrin and 5000 U/Kg EPO i.p in two independent injections. For irradiation, animals were placed under anaesthesia using 3% isofluorane (Cat.# 60302; PPC, Canada) in oxygen, and maintained at 1% isofluorane. A 4 by 6 mm elliptic craniotomy was performed to expose the dura, approximately 2.5 mm medial to the left and 5 mm caudal from the bregma. Special
care was placed not to injure the dura, to avoid bleeding. Using a stereotactic holder, a 2 mm diameter plastic fibre was positioned in contact with the dura and used for illumination with a 635 nm diode laser with a power output at the end of the fibre of 20 mW. A cut-end, 140 µm outer diameter, polyimide coated, silica-silica fibre protruding 2.5 mm and adjacent to the light delivery fibre was inserted 2.5 mm into the brain at the most caudal position as a means of measuring the fluence rate. Light coming from this fibre was collected with a photodiode and the voltage (proportional to fluence rate) was integrated in time until the total prescribed dose was achieved (2J/cm² measured at 2.5 mm in depth). Animals were sacrificed 24 hr later with an intra-cardiac injection of 0.2 ml pentobarbital (Euthanyl 240mg/ml) given under 5% isofluorane anaesthesia. Brains were removed immediately and placed in ice-cold PBS prior to sectioning and staining for viability.

4.2.7 Determination of tissue necrosis

Viability staining with triphenyltetrazolium chloride (TTC, Cat.# T8877; Sigma) was carried out according to standard protocols [Joshi et al. 2004]. Briefly, brains were placed in a rat brain matrix device (Ted Pella, USA) and 2 mm coronal sections were cut from the fresh tissue. These sections were then incubated in TTC 0.1% in PBS for 30 min at 37°C shaking every 10 minutes to ensure proper access of the solution to all surfaces. Sections were rinsed once with PBS and fixed with 5% neutral buffered formalin for at least 2 hours prior to imaging. Sections preserve contrast for at least 48 hr. Imaging was carried out using a flat-bed scanner at a 600 DPI resolution. Sections were placed between two sheets of transparency film and both sides were recorded. To determine the volume of necrosis, first the green channel was extracted to gain contrast, the area of necrosis for each face was measured using ImageJ (http://rsb.info.nih.gov/ij) and multiplied by half the thickness of the slice (i.e. 1 mm). All the face volumes were subsequently added to find the total volume per brain.
4.2.8 Immunocytochemistry

Cultures were carefully rinsed twice with PBS and fixed with 4% formaldehyde (prepared fresh from para-formaldehyde in PBS) for 10 min RT, and later kept in PBS at 4°C until use. Cells were permeabilized for 30 min RT with 5% Triton-x in blocking solution (4% BSA, Cat.#ALB002; Bioshop, Canada; 2% goat serum, Cat.# 16210-064; Invitrogen), and blocked overnight at 4°C. Incubation with primary EPOR antibody (1:50 dilution) was performed overnight at 4°C, followed by three 5min washes in PBS, and an 1 hr RT incubation with biotin-conjugated anti-rabbit antibody (3µl/ml Cat.#BA1000; Vector Laboratories). After washing 3 times with 0.1% NP-40 in PBS, labelling was done with FITC-avidin conjugate (1:250, Cat.# A-2001; Vector Laboratories) and Hoechst 3342 (1µM, Cat.# B 2261; Sigma) for 10 min RT. Coverslips were then gently rinsed twice with PBS and mounted on slides. Secondary antibody specificity was verified by omitting the primary antibody.

4.2.9 TUNEL staining

TUNEL staining (FragEL DNA Fragmentation Detection Kit; Cat. # QIA39; Calbiochem) of primary cortical neurons plated on 48 well plates was carried out using the vendor’s protocol for staining of cell preparations fixed on slides. Cells were incubated for 1.5 h at 37°C with the TdT reaction mixture. Nuclei were labelled using Hoechst 33342 (1 µg/ml, Cat.# B2261; Sigma). Number of TUNEL positive nuclei and total number of nuclei were counted manually in two independent wells and three fields of view per well. At least 200 nuclei were counted per condition.

4.3 Results

Primary cortical neurons grown in Neurobasal medium supplemented with B27 displayed normal morphology. By day 5 in vitro, extensive dendritic trees had been formed. This
morphology was still present by day 10 in vitro (fig. 4.2), when glutamate exposure took place. It is worth noting that neurons were extremely sensitive to changes in pH and temperature, full replacement of the growth medium, and when the growth surface was not sufficiently adherent. These changes rapidly led to loss of neurites and cell death. Baseline survival observed by day 10 was between 75 and 85% as determined by Sytox uptake.

**Figure 4.2** – Dark field micrograph of primary cortical neurons 10 days after plating. Notice the extensive dendritic trees and the absence of signs of degeneration. Scale bar=30µm

### 4.3.1 EPOR is expressed in primary cortical cultures

To verify that neurons expressed EPOR, Western blotting and immunocytochemistry was performed on cultured neurons with an EPOR antibody that targets the C-terminus cytoplasmic domain (fig. 4.3). **Figure 4.3 A** shows EPOR protein expression for cultured cortical neurons (lane 2). Two positive controls were included: EPOR overexpressing cells (BaF3-EPOR, lane 1) and fetal rat liver (lane 3). BaF3 cells with no EPOR expression are also shown (lane 4). While neurons and fetal liver do not display the 64kDa EPOR
band (lower arrow) seen in lane 1, a second band at 70 kDa (upper arrow) can be clearly seen in lanes 1 through 3, but not in lane 4, consistent with EPOR immunoreactivity. Immunocytochemistry revealed a population of neuron-like cells with diffuse immunoreactivity of cell bodies and processes (fig. 4.3B), that was not seen if the primary antibody was omitted (fig. 4.3C).

**Figure 4.3** – EPOR is expressed in primary cortical cultures. (A) Western Blotting of BaF3-EPOR cells (lane 1), cultured cortical neurons (lane 2), fetal rat liver (lane 3), and BaF3 cells (lane 4). Lanes 1 and 3 are positive controls and lane 4 is a negative. Although the expected 64 kDa product (lower arrow, lane 1) is not observed in neurons or fetal liver, a 70 kDa band is seen in lanes 1-3 but not in the negative control (lane 4). (B) Micrographs of cultured cortical neurons stained for EPOR (green) and DNA (blue). (C) Primary antibody omitted. Scale bar=30µm.

### 4.3.2 EPO does not change the viability of glioma cell lines treated with PDT

To establish whether EPO selectively conferred protection from PDT to neurons versus tumour cells, a panel of glioma cell lines was treated with ALA and Photofrin mediated PDT in the presence or absence of EPO (5 IU/ml for 24 hr prior to irradiation). Viability was assayed using resazurin, 24 hr post light delivery (fig. 4.4). No difference
in survival was observed between groups with or without EPO, suggesting that EPO mediated protection is potentially selective.

Figure 4.4 – Effects of EPO on glioma cell lines treated with ALA and Photofrin mediated PDT. Viability in the presence (---) or absence (—) of EPO of glioma cell lines: CNS-1 rat astrocytoma (○), 9L rat gliosarcoma (×), and U87 human glioblastoma (+). Both ALA (A) and Photofrin (B) mediated PDT were tested. Each point is the average of three independent determinations. The error bars have been omitted for clarity but they overlap for all EPO treated and untreated data points.

4.3.3 EPO fails to protect primary cortical neurons treated with ALA mediated PDT

The ability of EPO to protect neurons treated with ALA mediated PDT was tested (fig. 4.5 A). Following an 18 hr incubation with 10 IU/ml EPO, neurons were incubated in medium containing 100 or 200 µg/ml ALA for 4 hr, and immediately irradiated for up to 45 min (in fresh medium). Cell viability was assayed 24 hr later.

Administration of EPO did not seem to improve survival from cell death induced by ALA mediated PDT. Similar results in terms of EPO pre-treatment were observed
with Photofrin mediated PDT and using red light (600-700 nm) instead of white light; however, less cell kill was evidenced with red light for the same irradiation times. No difference between using 100 or 200 µg/ml ALA was evidenced, consistent with ALA being supplied in excess.

4.3.4 Survival of primary cortical neurons after various insults used as positive control for EPO mediated protection

Following the lack of an effect of EPO with PDT, various challenges were evaluated as positive controls of EPO mediated protection (fig. 4.5 B-H). 10 day old cultures were treated for 18 hr with or without 10 IU/ml EPO and then incubated with glutamate at the concentrations and times indicated. Viability was assessed 24 hr later using the Sytox or resazurin methods, as indicated.

After averaging the results of cultures derived from 4 different rats, no evidence was observed that EPO mediates neuronal protection from glutamate induced excitotoxicity (fig. 4.5 B). Given the high neuronal viability observed following a 300 µM glutamate challenge (typically sufficient to kill most neurons in culture), the dose response for up to 3 mM glutamate was also measured under the same conditions (fig. 4.5 C). The results show that relative survival after 300 µM glutamate plateaus at around 70% (approx. 60% absolute survival) and that EPO pre-incubation has no effect. This verified that the dose range considered was adequate.

Since a 1.5 hr glutamate challenge likely causes cell death primarily via necrosis, thereby curtailing EPO’s ability to exert protection, 15 min and 2 hr glutamate challenges were also tested (fig. 4.5 D). The extent of cell kill is similar between both incubation times, and it is consistent with the previous glutamate challenges. With the 15 min glutamate exposure, EPO does not improve survival. The improved survival with the 2 hr glutamate exposure (evident with 300 µM glutamate) is in fact an artifact caused by the low survival of the no glutamate no EPO control (notice the large standard deviation).
Figure 4.5 – Exposure of neurons to various challenges
**Figure 4.5** – (Cont.) Effects of 18 hr incubation with 10 IU/ml EPO followed by various challenges: ALA mediated PDT (A), glutamate excitotoxicity (B-D), staurosporine (E,G), and camptothecin (F,G). All assays were carried out 24 hr after challenge. (A) Cultures were treated with PDT at various ALA concentrations and light doses as indicated. Survival was measured using Sytox. (B) Glutamate was given for 1.5 hours and viability was measured using resazurin. (C) Extended dose response following 1.5 hr glutamate exposure and survival measured using Sytox. (D) Glutamate exposure was carried out for 15 or 120 min and measured using Sytox. Cultures were exposed for 24 hr to staurosporine (E) or camptothecin (F) and survival was measured using Sytox. (G) Ratio of TUNEL positive cells in untreated cultures (CTR), those receiving 0.5% DMSO (VEH), or treated with various concentrations of staurosporine (SP) or camptothecin (CT). Error bars were derived from Poisson statistics (STD=$\sqrt{n}$). (g) Micrograph of TUNEL stained cultures treated with 10µM camptothecin and no EPO. Notice that some apoptotic nuclei are not TUNEL positive. Except for panel (B), where 4 different cultures were averaged (mean±SEM, N=4), all others panels report the averages for one culture with at least 6 wells averaged per condition (mean±STD).
In addition to these experiments, the conditions of EPO incubation were also varied (not shown). Incubation times spanning 0 to 24 hours and concentrations ranging from 5 to 100 U/ml EPO were tested with similar negative results.

Following the hypothesis that for EPO to effectively confer protection the insult has to effect cell death via apoptosis, we tested the classical apoptotic inducers staurosporine and camptothecin. In multiple trials (two of which are shown in figures 4.5E and 4.5F) administration of EPO was unable to improve the survival of neurons from these challenges. Furthermore, TUNEL staining revealed apoptotic indices similar to the overall cell kill observed, corroborating that indeed these insults are mostly apoptotic in nature (fig. 4.5G). Importantly, a protective effect was seen with the camptothecin challenge, were EPO administration seems to reduce the incidence of apoptosis. This suggest that under these conditions EPO is in fact able to confer protection from camptothecin mediated apoptosis. However, notice that this reduction in apoptosis does not seem to translate to overall survival (fig. 4.5F).

4.3.5 EPO fails to reduce the volume of PDT induced necrosis in the rat brain

To directly test the ability of EPO to protect normal brain from PDT, animals were treated with Photofrin mediated PDT in the presence or absence of EPO. The volume of necrosis was then measured 24 hr following light delivery. This early time point accounts primarily for direct cell kill caused by PDT. The photosensitizer Photofrin was chosen for this in vivo study because it results in more reproducible lesions. Reproducibility was further improved by interstitially measuring the fluence rate and irradiating until a target fluence was achieved.

Light was delivered through the intact dura resulting in lesions largely confined to the cortex (fig. 4.6A). Measuring the size of these lesions revealed that administration
of EPO 24 hr prior to light delivery did not reduce the volume of PDT induced necrosis, as seen in fig. 4.6B.

Figure 4.6 – EPO fails to reduce the volume of PDT induced necrosis. (A) Typical TTC-stained axial slices used for volume quantification. Rostral and caudal aspects of each section are shown side by side. (B) Volume of necrosis with or without EPO. The mean and standard deviation are shown next to each treatment group. (C) Relationship between the volume of necrosis and the irradiation time.

Figure 4.6C shows a weak correlation between the volume of necrosis and the irradiation time (Spearman’s correlation coefficient $\rho = 0.58, P = 0.052$). However, irradiation times did not differ significantly between groups (Wilcoxon rank sum test for different medians: $P = 0.88$), and therefore, no bias was present towards either group.

4.4 Discussion

The purpose of this study was to determine the feasibility of using EPO in order to protect normal brain tissue from PDT toxicity. This is the first step toward the goal of using EPO as an adjuvant to PDT of brain tumours in order to increase the therapeutic efficacy of the treatment. \textit{In vivo}, EPO did not reduce the volume of necrosis induced by Photofrin mediated PDT of normal rat brain, measured 24 hr after light delivery. \textit{In
vitro, the results largely suggest that EPO does not confer protection from ALA mediated PDT of cultured primary cortical neurons, and thus support the in vivo findings. To the best of our knowledge, this is the first study testing EPO mediated neuroprotection in combination with PDT.

Different photosensitizers were chosen for the in vivo and the in vitro studies. ALA was chosen for the in vitro work for its ability to kill cells via apoptosis [Kriska et al. 2005, Uberriegler et al. 1995, Webber et al. 1996]. Photofrin was administered in the in vivo experiments because of its widespread use in clinical trials of brain PDT [Stylli & Kaye 2006b, Muller & Wilson 2006]. Additionally, Photofrin mediated PDT causes brain lesions that tend to be more reproducible than with ALA mediated PDT, making the lesion volume easier to quantify.

To validate the in vitro results with PDT, I attempted to reproduce results from the literature were EPO mediated neuroprotection had been verified. Glutamate and NMDA mediated excitotoxicity were chosen as such positive controls. The results for NMDA are not reported here, but the conclusions drawn from them mirror those of the glutamate studies. Despite testing multiple EPO and glutamate dosages, the findings of this study strongly differ from those of Morishita et al. [1997], where they demonstrate EPO mediated neuroprotection from glutamate excitotoxicity under similar conditions (figs. 4.5 B-D). The authors use a 15 min glutamate challenge that resulted in lower neuronal survival than the one reported here (approximately 40% vs 60% survival). Critically, the authors found that survival in EPO treated groups was nearly restored to baseline. Other studies using glutamate and EPO have contrasting results. On the one hand, Yamasaki et al. [2005] report that 1.5 IU/ml of EPO restore survival of retinal ganglion cells after treatment with glutamate for 24 hours. On the other hand, Sinor & Greenberg [2000] and Kawakami et al. [2001] found that EPO had no neuroprotective effects against glutamate in cortical neurons and organotypical cultures, respectively. Together with our results,
these studies suggest that EPO mediated protection from glutamate is highly context dependent.

The discrepancies in EPO mediated neuroprotection from glutamate in vitro could be due to several reasons: EPO and EPOR signalling status; EPO dosage; glutamate treatment leading to different cell death mechanisms; and differences in culture systems. As a first step, eventhough EPO quality-tested for human use was used, we outruled the loss of EPO activity by testing the ability of EPO to support the growth of BaF3-EPOR cells, which require EPO to survive (data not shown). Additionally, EPOR expression was verified by Western blotting and immunocytochemistry in neuronal cultures (fig. 4.3). A 70 kDa band consistent with EPOR was identified in neuronal cultures. A band of the same mass was observed by Kirkeby et al. [2007] in rat brain tissue homogenates. We did not verify the identity of the 70 kDa band, but this could be carried out with an EPOR blocking peptide in competition assays. It is interesting that the rat tissues show strong immunoreactivity at this band and not at the expected 64 kDa mass. The greater apparent mass is likely due to differences in protein glycosilation or phosphorilation [Sawyer & Hankins 1993], and to differences in post-translational processing between species. Immunocytochemistry revealed a largely cytoplasmic antibody immunoreactivity suggesting that, while the protein is being produced, most of it is found in transit to the cell membrane. This latter observation agrees with the subcellular distribution of EPOR in haematopoietic cells, where more than 90% of the protein is present in the endoplasmic reticulum and the Golgi [Lodish et al. 1995, Hilton et al. 1995]. Ultimately, one wishes to show signalling through EPOR. Phosphorilated JAK2 would be a first candidate to test, since it is a critical event following binding of EPO by EPOR. However the presence of other cytokines that also signal through JAK2 can mask this test. Other possible downstream endpoints that could be tested are Akt phosphorilation [Chong et al. 2003a] and NFκB translocation to the nucleus [Digicaylioglu & Lipton 2001].
The most salient difference in the experimental conditions between Morishita et al. [1997] and this study, was that the EPO concentrations used by Morishita et al. [1997] were 10 to 100 fold less than those used by us (0.01-0.1 vs. 10 IU/ml EPO). While it is possible that we entirely missed the dose range required for EPO mediated protection from glutamate, as only concentrations from 5 to 100 IU/ml were tested, multiple studies with other challenges find 0.1-10 IU/ml an optimal range for in vitro protection [Chong et al. 2003b, Leist et al. 2004, Sirén et al. 2001, Digicyałyoğlu et al. 2004]. This dosage discrepancy can potentially be verified by extending the dose range of this study.

EPO neuroprotection is mediated by inhibiting apoptosis, with multiple complementary anti-apoptotic pathways being activated simultaneously [Brines & Cerami 2005, Li et al. 2004, Weishaupt et al. 2004]. Furthermore, EPO does not seem to provide protection against insults that cause necrosis. Bonfoco et al. [1995] have shown, for NMDA mediated excitotoxicity and nitrosative stress, that mild versus intense insults result in apoptotic, respectively necrotic neuronal demise. I investigated a range of glutamate concentrations (fig. 4.5D) and incubation times (fig. 4.5D) to determine whether the severity of the insult had an effect on EPO mediated neuroprotection, but no such trend was observed. Further pursuing this hypothesis, staurosporine and camptothecin were used to induced apoptosis. For these insults, while extensive apoptosis induction was confirmed using TUNEL (fig. 4.5G), no improvement was seen in the survival of EPO treated cultures 24 hr after challenge (figs. 4.5E,F). EPO mediated protection from staurosporine has been observed in SH-SY5Y neuronal cell lines [Pregi et al. 2006] and in cardiomyocytes [Brines et al. 2004, Fiordaliso et al. 2005]; however, protection has been only reported as a decrease of the apoptotic index (often as early as 16 hr post challenge). While we did not observe that EPO reduced the number of TUNEL positive neurons for staurosporine 24 hr after challenge, we did see protection from camptothecin. The differences in the levels of protection are very likely due to the dynamics of apoptotic cell death and, therefore, depend strongly on the time points selected for the assay. Stau-
Rosporine can initiate apoptosis as early as 4 hr [McKeague et al. 2003], thus requiring that apoptosis be measured relatively early, or otherwise any delay in the incidence of apoptosis would be missed. Camptothecin mediated apoptosis develops slower, with inter nucleosomal cleavage still occurring at 24 hr [Lesuisse & Martin 2002]. Notice, however, that scoring apoptosis as a surrogate for survival is inadequate since it only addresses changes in the kinetics of cell death, and does not necessarily reflect overall survival improvement. In conclusion, while EPO did seem to reduce the incidence of camptothecin induced apoptosis at 24 hr, demonstrating that EPO did in fact had an effect on cultured neurons, this did not translate into any differences in terms of survival.

Finally, differences in culture conditions can modulate the sensitivity to growth factors and cytokines [Fink et al. 1996, Price et al. 2005]. The present study uses Neurobasal medium supplemented with B27, the same used by Morishita et al. [1997]. However, this formulation, originally designed by Brewer et al. [1993], has been recently modified by the manufacturers and the components are no longer known. It is possible that the inclusion of growth factors to better support neuronal survival may be masking any EPO mediated neuroprotection. One could try to incubate without B27 during EPO pre-treatment to reduce the presence of other growth factors, but neurons are highly sensitive to such drastic changes in growth conditions. The lack of an effect displayed by EPO in most conditions and challenges tested highly suggests that EPO mediated protection is not as robust as we initially hypothesized, and that it is highly culture system and challenge dependent.

Regardless of artifacts associated with the in vitro studies, EPO failed to reduce the volume of necrosis directly caused by Photofrin mediated PDT of normal rat brain (fig. 4.6). Necrosis was assayed 24 hr after photoactivation because this time-point reflects direct PDT cell kill rather than secondary cell death that appears several days after light delivery due to inflammation and ischemia [Yoshida et al. 1992a,b]. At this early time-point, the depth of necrosis for PDT induced lesions depends roughly with the logarithm
of the light dose [Dereski et al. 1991, Lilge et al. 1996]. Because of this logarithmic behaviour, only very large reductions in the sensitivity of normal brain to PDT due to EPO would appreciably decrease the volume of necrosis. In other words, because of the steepness of the light dose gradients, differences in the volume of necrosis can only occur if EPO mediated protection is very strong. Such levels of protections were not observed in this study at this time point. This suggests that using EPO in combination with PDT can be more promising in illumination geometries that display less steep gradients, such as surface illumination, or where normal brain and tumour coexist at the same depth. PDT of age-related macular degeneration (AMD), unquestionably the most fecund use of PDT in medicine, satisfies these geometrical requirements. Testing of EPO as an adjuvant to PDT for AMD seems a promising endeavour, particularly given EPO’s ability to protect retinal tissues [Kilic et al. 2005b, Weishaupt et al. 2004, Yamasaki et al. 2005] and recent studies suggesting the use of neuroprotectants in combination with PDT for AMD (primarily brain-derived neurotrophic factor BDNF) [Paskowitz et al. 2007, 2004]. In addition, in human patients, the methods of Chapter 2 can potentially be used to improve the uniformity of the light dose making the requirements for neuroprotection less stringent.

The observations by Yoshida et al. [1992a,b] that identified a second wave of neuronal death seven days after PDT offer another opportunity for EPO to exert protection at later time points. EPO is known to protect from inflammation and other secondary effects following brain injury [Lee et al. 2006, Brines et al. 2004, Chong et al. 2003a]. Therefore, a protracted EPO regime, while unable to protect from the primary insult, can reduce the overall damage to the brain at later time-points. This was indeed the finding for radio-surgically induced brain lesions, where no EPO mediated protection was observed in the initial stages of the evolution of the lesion, yet, 90 days after treatment, a 50% reduction in the volume of the lesion was noted [Erbayraktar et al. 2006]. Future
work should extend the time window to observe EPO mediated protection in the context of brain PDT.

Basal EPOR protein levels in the normal brain are very low. As a result of brain injury (e.g. hypoxia), the protein levels of EPOR increase, thereby making the tissue more responsive to EPO [Spandou et al. 2004]. This has two implications regarding the use of EPO in brain PDT. It can explain why little protection from the primary PDT insult was seen (both \textit{in vitro} and, specially, \textit{in vivo}), given that it is not a gradual insult but a sudden, disseminated, vascular shut-down. Importantly, it suggests a mechanism to improve EPO neuroprotection by pre-upregulating EPOR prior to EPO administration and PDT delivery. TNF-\(\alpha\) (tumour necrosis factor \(\alpha\)) has been shown to have this effect \textit{in vitro}. However, TNF-\(\alpha\) also mediates pro-inflammatory process after CNS injury, thus posing a dilemma for its use. IGF-I (insulin-like growth factor I) has been shown to synergistically potentiate EPO’s ability to mediate neuroprotection [Digicaylioglu et al. 2004]. It would be interesting to test whether certain dose regimes of these or other compounds in combination with EPO can be used to increase the therapeutic efficacy of PDT and other brain therapies.

Clearly, no improvement of the therapeutic index will be seen if EPO also protects tumour cells. Sinor \& Greenberg [2000] demonstrated that EPO does not protect astrocytes in culture from hypoxia and AMPA toxicity. We hypothesized that astrocytomas would equally be unaffected by EPO, and was verified for a panel of glioma cell lines (fig. 4.4). However, the validity of these results is put into question given the generalized lack of an effect for EPO with neurons \textit{in vitro}. Recently, Mittelbronn \textit{et al.} [2007] found that, while low grade gliomas produced higher levels of EPOR protein with respect to astrocytes, a significant decrease in the protein expression was observed as the grade of malignancy increased. Interestingly, higher than average EPOR expression in high grade gliomas was positively associated with longer survival. These results suggest, contrary to what would be expected for the receptor of a survival factor, that high EPOR expression
is beneficial in gliomas. The potential for tumour neuroprotection by EPO, or any other neuroprotective strategy, is certainly something that needs to be thoroughly tested in the course of this type of research. Critically, one needs to also determine if any such neuroprotective interventions can in fact enhance tumour growth.

In conclusion, early time-points, steep dose gradients, and the suddenness of the PDT insult have all contributed to masking any EPO mediated neuroprotection \textit{in vivo}. New PDT dosage strategies such as metronomic PDT [Bisland \textit{et al.} 2004] and protracted EPO regimes still remain a promising strategy to improve the treatment of brain tumours.
Chapter 5

Conclusions and future work

The primary goal of this thesis was to develop and test novel strategies to improve the therapeutic index of Photodynamic therapy without compromising its efficacy. The tissue’s biological response to PDT depends on the type and biodistribution of the photosensitizer, the light dose, the light dose rate, the oxygen availability, and the inherent tissue sensitivity. Therefore, all of these parameters can be used to modulate the efficacy of the treatment. However, it is less clear how they can be manipulated to improve PDT’s selectivity. The main-stream approach has been to develop new photosensitizers with improved selectivity and efficacy. In fact, few sensitizers have both properties. Furthermore, for interstitial PDT, it is critical that the photosensitizer has a long absorption wavelength. These requirements pose very stringent constraints on photosensitizer development.

I have chosen two strategies that complement the photosensitizer-based approach and, in principle, are photosensitizer independent. The first strategy was aimed to improve our ability to conform the light dose in interstitial PDT by using cylindrical diffusers with tailored emission profiles. This strategy is analogous to conformal radiation therapy, where the ability of conforming the dose to a treatment volume is improved by increasing the freedom of specifying the irradiation geometry. For PDT, conformal light delivery
is particularly advantageous because it enables using photosensitizers that may be very potent, but are otherwise poorly selective.

Other strategies to improve selectivity are required when treating tumours that are not well confined, such as in the case of gliomas. This is because our ability to shape the light dose will always fall short of the selectivity needed to treat a highly infiltrative disease. The second approach has focused on selectively modifying the normal tissue response to PDT for the treatment of malignant gliomas. By using neuroprotectants to reduce the sensitivity of normal tissues to PDT, the treatment can be made overall more selective. For this type of tumours, the higher the selectivity, the better are our chances to improve outcome without significantly increasing morbidity.

My results suggest that the strategies proposed here did not achieve a substantial improvement of the therapeutic index for the particular indications selected. Importantly, however, through this work, our understanding of the fields involved has advanced. Let us review the main findings and contributions of this thesis:

## 5.1 Principal findings and contributions

### 5.1.1 Conformal light delivery

1. **Prostate coverage** – The parameter that most strongly determines prostate coverage, without compromising the rectum, is the number of diffusers employed. Importantly, the improvement plateaus for increasing number of diffusers.

2. **Tailored vs conventional** – In comparison to conventional diffusers, tailored cylindrical diffusers offer only marginal improvement of the ability to conform the light dose to the prostate, particularly when the length and \( z \)-position of the diffusers are also optimized.
3. **Optimization algorithm** – In order to achieve the previous conclusion, I developed an optimization based treatment planning platform. Central to this treatment planning platform is the introduction of a feasibility algorithm that improves upon existing ones by ensuring adequate convergence and regularization of the solutions.

4. **Spatial MTF** – I derived a mathematical formulation for the loss of modulation of the contour lines arising from a tailored diffuser as they propagate through tissue. This provided an explanation to the results mentioned in item 1. It also demonstrated that the shape of the light dose distribution is relatively insensitive to changes in optical properties, a result that was also verified by a sensitivity analysis. This has important consequences because it emphasizes the potential of combining pre-treatment planning with on-line monitoring for performing light dosimetry in PDT.

5. **Placement uncertainty** – Small uncertainties in diffuser placement do not affect prostate coverage substantially. This is important because such uncertainties are commonplace in the clinic, and this finding suggests that exact diffuser placement is not critical.

### 5.1.2 Selective neuroprotection

1. **EPO neuroprotection** – Erythropoietin did not confer any significant protection to normal rat brain treated with Photofrin mediated PDT, 24 hours after light delivery.

2. **In vitro studies** – EPO mediated neuroprotection *in vitro* is highly context dependent, even for a well established insult such as glutamate mediated excitotoxicity. Furthermore, one must exercise care when extrapolating results from other mechanisms of cellular injury to PDT.
5.2 Assumptions, limitations and scope

5.2.1 Conformal light delivery

- **Homogeneous optical properties** – Throughout this work, I have assumed that the optical properties of the tissue are homogeneous. This is far from true [Chen et al. 1997, Zhu et al. 2005a, Svensson et al. 2007], and heterogeneity in the optical properties does have consequences in terms of the shape of the light dose distribution [Li & Zhu 2007]. The main implication of this assumption, and thus its utility, is that the light dose arising from a diffuser could be expressed as the superposition of point sources. More technically, this permitted an efficient computation of the light dose distribution, which is needed for the optimization algorithm. In addition, when analyzing the sensitivity of the light dose distributions to changes in optical properties, the optical properties were also varied homogeneously. This assumption, however, is difficult to remove because there is a lack of efficient methods to calculate the light dose in heterogeneous conditions.

- **Biological response model** – I have used here a threshold model for the biological response to PDT [Patterson et al. 1990]. For dosimetry purposes, I have assumed three things: (I) the photosensitizer is homogeneously distributed throughout the organ; (II) the light dose (total fluence), rather than all parameters affecting the PDT dose, is sufficient to calculate the PDT dose; and (III) the biological response to that PDT dose is all or nothing. There are many reports highlighting the pitfalls of these assumptions. Photosensitizer distribution throughout the prostate is not homogeneous [Zhu et al. 2005b]. Furthermore, not only the light dose, but also the light dose rate (fluence rate) are important in the photodynamic process, and drastically affect its efficacy [Iinuma et al. 1999, Henderson et al. 2006]. Finally, the biological response does not necessarily need to be thresholded for all tissues
Chapter 5. Conclusions and future work

[Lilge et al. 2000], although it is a common observation in prostate PDT [Martin & Hahn 2004].

- **Limited clinical input** – Photodynamic therapy for prostate is in its infancy. Our optimization platform requires a variety of inputs derived from clinical experience, particularly, the threshold doses for each organ and the variation of those thresholds between patients. Such information is at best incomplete. I derived the threshold values based on preliminary findings for WST09 mediated PDT [Trachtenberg et al. 2007].

Additionally, to estimate the therapeutic index, one has to have a good sense of the TCP and NTCP. There is little known about them beyond anecdotal observations. It is difficult to make extrapolations from other modalities such as radiation therapy and brachytherapy because these modalities because of their longer penetration depths and their different mechanisms of action resulting in late rather than immediate complications, as is the case of PDT. This is particularly problematic for establishing a good measure of rectal complications. Here, I have simply used differences in the DVHs. More clinical information will become available as clinical trials progress.

5.2.2 **Selective neuroprotection**

- **EPO selectivity** – Only normal rat brain was used in this study because the goal was to establish whether there was a potential for EPO to improve the selectivity of PDT. Clearly, to test selectivity one needs to perform these assays in tumours as well. I performed preliminary *in vitro* studies with three different glioma cell lines, and showed that EPO did not affect the viability of cells treated with ALA and Photofrin mediated PDT (fig. 4.4). These early experiments suggested that indeed there was a rationale for the selectivity of EPO’s neuroprotection. In light of the
subsequent *in vitro* studies on cultured cortical neurons, the applicability of these results to the *in vivo* situation remains questionable.

- **Early time point and functional studies** – I only tested EPO’s ability to protect normal brain 24 hours after light delivery. This is an appropriate time point to determine whether the volume of necrosis has been reduced [Dereski *et al.* 1991, *Chen et al.* 1996]. It also provides a direct test for the reduction of the threshold value for necrosis. However, 24 hours completely misses secondary waves of neuronal death [Yoshida *et al.* 1992a], and possibly EPO’s ability to modulate the healing process (i.e. “clean-up” followed by revascularization and neurogenesis [Iwai *et al.* 2007, *Kolb et al.* 2007]). These secondary processes are best measured with functional tests for neurologic function [Li *et al.* 2007, Villa *et al.* 2007].

- **Potential as a growth factor** – It is important to highlight that EPO has roles in development and differentiation [Buemi *et al.* 2003, Juul 2000], often acting as an inhibitor of apoptosis and as a growth factor Fisher [2003]. It is therefore important to establish whether EPO can potentially enhance tumour growth. While some reports suggest that this is not the case [Hardee *et al.* 2005], any effects are most likely cancer dependent. This is a limitation that strategies involving growth-factor mediated neuroprotection must consider.

### 5.3 Future avenues

#### 5.3.1 Conformal light delivery

- **Other geometries** – The next step is to determine whether tailored diffusers can improve the light dose distribution in more complex geometries. The prostate is a relatively simple organ in terms of its shape. Furthermore, most of the anatomical structures that one needs to avoid run parallel to the long axis of the diffusers.
This arrangement is not the best suited to test whether tailored diffusers can bring about improvement. Other interstitial locations need to be tested before discarding tailored diffusers for light delivery. One such condition is head and neck cancer that often present with complicated shapes in close proximity to important structures such as the eyes or various nerves [Lou et al. 2004]

- **Heterogeneity** – An important aspect that needs to be examined more carefully is to what extent assuming homogeneity changes the light dose distribution. Such research would help elucidate the origin of the variability in treatment response between patients, a fact that currently remains largely unexplained. This can be explored at two different levels. One can study the effects of heterogeneous changes in the optical properties relative to a treatment plan that assumes constant values throughout the tissue. This requires solving the transport equation with spatially varying optical properties. Finite element methods are well suited for this kind of proposition. The Lund group has began studying these effects with a similar approach for their PDT treatment platform [Johansson et al. 2007a].

If indeed such studies show that heterogeneous optical properties lead to important changes in the light dose distribution, one should consider finding strategies to solve the optimization task (that is, the pre-treatment planning) taking into consideration the heterogeneities involved. This poses serious computational challenges since finite element methods are too slow for optimization based tasks, which require at least thousands of calculations of the light dose. How to resolve this dilemma remains unclear; perhaps perturbation methods\(^1\) can help speed up the calculations, while still remaining accurate enough to provide an improvement over the existing homogeneous methods.

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\(^1\)Perturbation methods are a general strategy to account for heterogeneities, where small modifications in the optical properties are added to an otherwise constant set of optical properties [Ostermeyer & Jacques 1997]
• **Integration with on-line dosimetry** – Ultimately, our knowledge of the optical properties is and will remain limited, and thus, on-line dose monitoring will always be necessary. In consequence, there is a strong need to find strategies to best combine pre-treatment planning and on-line monitoring, with the possibility of using the dose monitoring to provide feedback for optimization algorithms. This poses many interesting challenges from the technical, computational, and clinical perspectives. For example, one would like to know where to put measuring probes in order to best interpolate the light dose elsewhere; how to make the optimization algorithm faster and more stable; and what dosimetry models can be used to predict treatment outcome more accurately.

• **Uniform interstitial illumination** For highly infiltrative cancers such as gliomas, conformal light delivery cannot offer sufficient selectivity to effectively target the disease. Furthermore, as already discussed in Chapter 4, the steep light dose gradients degrade the selectivity due to the photosensitizer, or in our case, the neuroprotectant. One can use the methods of treatment planning optimization to improve the uniformity of the light dose, thus reducing the steepness of the gradients and improve the treatments selectivity.

### 5.3.2 Selective neuroprotection

• **Other neuroprotectants** – The idea of improving the TI by selectively reducing normal tissue sensitivity is worth pursuing with other neuroprotectants. One would mirror the *in vivo* studies presented here with the important addition of later observation time points and assays for neurological function. A first candidate could be EPO, administered for several days after PDT possibly in combination with other compounds. Other possible approaches include several growth factors, or more radically, hypothermia [den Hertog et al. 2007] or osmotherapy [Ziai et al. 2007].
The neuroscience literature has plenty of different options that one could implement; however, it is unclear how to extrapolate results from other kinds of insults to PDT, and which neuroprotective interventions are potentially selective towards normal tissue.

- **Other conditions** – One can use tissue protective strategies for other diseases such as aged-related macular degeneration (AMD) that, because of their planar geometry, can potentially be better suited for this kind of approach. In planar geometries, the light dose gradients are less steep; and therefore, the requirements for the degree of neuroprotection are less stringent. In AMD, blood vessels resulting from neovascularization of the retina, are in the same plane relative to the direction of light propagation. This relaxes the required degree of neuroprotection and offers a great opportunity to test our strategy to improve the TI. In fact, this approach has already been suggested for several other neurotrophic factors [Paskowitz *et al.* 2004, 2007].

## 5.4 Relevance of these studies

Let me conclude this dissertation with my perspectives about the challenges and future of PDT in oncology.

Let us begin by asking what can PDT offer over other cancer treatment modalities? Firstly, there is the absence of mutagenic side-effects so prevalent in chemotherapy and radiotherapy. In fact, the lack of mutagenic side-effects enables re-treatment with PDT as frequently as necessary, without the accumulation of normal tissue toxicity over time. Secondly, with current fibre optic-based technologies, PDT, even interstitially, is a minimally invasive intervention which could potentially replace surgery for some locations that are difficult to access or even inoperable. Thirdly, at least in principle, PDT has the exquisite ability to combine two levels of selectivity. The first level arises from the
preferential uptake of the PS by the tumour, with several strategies to further target the photosensitizer to the tumour currently underway. This selectivity is further enhanced by low penetration of light in tissue which potentially can be used to confine light to precise volumes. Thus PDT is, first and foremost, a local type of treatment.

So why after 30 years of clinical trials and over 100 years since its origins, has PDT not become more mainstream? Well, certainly, it is still young in comparison with more conventional cancer therapies. But also, there is perhaps another face to its double selectivity. Photosensitizer delivery suffers from the same challenges that chemotherapy encounters in terms of drug biodistribution. This results in variable photosensitizer concentrations throughout a tumour. Furthermore, as opposed to radiation therapy, where predicting the radiation dose is relatively simple, since soft tissues have a fairly homogeneous attenuation coefficient at those wavelengths, PDT requires irradiation at wavelengths where things are in colour (granted, mostly shades of red). This means that large changes in the light attenuation coefficient are common and occur dynamically. Moreover, tumour hypoxia, well known to degrade the efficacy of radiation therapy, has even stronger effects in PDT, given its strong dependence on oxygen.

And so, the potentially exquisite selectivity of PDT comes at the cost of extremely complicated dosimetry. This means that in order to perform accurate dosimetry one needs to spatially resolve a great number of parameters, and that is only to provide the data necessary to provide input to a biological response model. Let me highlight one critical implication. In order to compare the results from different patients and also from different clinical trials, one needs to understand the conditions under which PDT was delivered to these patients. But if PDT dosimetry is so involved, it becomes difficult to make these comparisons, and therefore, clinical knowledge becomes anecdotal. This, in turn, slows down the advancement of PDT. Of course, this is a rather pessimistic portrait of the situation, partially serving a rhetoric purpose, and significant advances have been indeed achieved for some cancers.
What should we do then? Where should our future efforts be placed? They should be placed in simplifying the dosimetry. That is why the threshold model is so attractive. There are at least three ways of simplifying the dosimetry. The first one, and perhaps the most widely applicable, is to find surrogate markers that are good predictors of treatment outcome and that can be measured during PDT delivery. This is for example the goals of singlet oxygen luminescence detection [Jarvi et al. 2006] and of vascular imaging during PDT [Standish et al. 2007, Gross et al. 2003]. It is, more generally, the goal of implicit dosimetry [Wilson et al. 1997]. With such approaches, one can forgo the measurement of all the other explicit dosimetry parameters and focus on a single one, or at least just a few.

The other two approaches go back to the first figure of this thesis. The idea is to separate the TCP and NTCP curves as far as possible. A first approach is to develop photosensitizers that are extremely selective and at the same time efficacious. Our neuro-protective strategy is closely associated with this type of selectivity. Moreover, It is this kind of selectivity that is needed to treat infiltrative diseases such a malignant gliomas. Light delivery strategies that improve uniformity can further improve the outcome in these situations.

But if the tumour is well confined, there is perhaps an easier approach. This involves choosing potent photosensitizers that do not need to be selective, but that have reproducible tissue distribution, and importantly, that absorbs as far as possible in the infrared (within the optical penetration window and the constraints placed by the activation energy of singlet oxygen). In this wavelength region the variations of the tissue optical properties are less dramatic and penetration depths larger. This may be for example the case of vascular acting sensitizers such as WST09. Additionally, the availability of oxygen may be less critical for vascular photosensitizers. Then, one can focus on careful pre-treatment planning, a very successful endeavour in radiation therapy, with the added benefit of the ability to perform on-line light dose monitoring. For this approach,
the relevance of my research in conformal light delivery becomes evident. Under these conditions, one would have reduced the complexity of dosimetry and achieved a highly localized, truly minimally invasive treatment modality, which fulfils the promises made at the beginning of this section.
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