

CHAPTER 5: EFFECT OF THE OPTICAL PROPERTIES OF THE EPIDERMIS ON LASER TREATMENT PARAMETERS

For most laser treatments the target site is some distance into the skin. The thickness of the epidermal layer in skin varies with location across the body. The absorption coefficient is a measure of the absorption (attenuation) of light through the medium (skin). The penetration depth of light in skin is determined by the total attenuation, constituted by the scattering and absorption coefficients. It stands to reason that a thicker epidermis will absorb more light than thinner epidermal layers. In determining the fluence rate reaching a specific position (depth) in the skin, both the epidermal thickness and the absorption coefficient need to be taken into account. The computer model developed and described in Chapter 3 was adapted to account for the different skin layers. Photodynamic Therapy (PDT), a potential treatment for skin cancer, was the application chosen to demonstrate the influence of the epidermis on the fluence rate in skin.

The aim of the model was to be as uncomplicated as possible to evaluate the influence of specific parameters. In the development of the model only the essential skin layers were used, i.e. the epidermis and dermis and no hair or blood vessels were included. The main reason for the model development is to quantify the expected fluence rate losses in the tissue before reaching the treatment site, in this case the tumour. The major contributions to the loss of fluence rate through the tissue are the absorption in the epidermis and the scattering of the light. Scattering of light leads to an increase in the spot size and as such reduces the ‘power density’ or fluence rate deeper in the skin. Consequently the loss in laser light intensity due to absorption depends on both the absorption coefficient and the path length through the medium (in this case the epidermis). As such the epidermal thickness is an important parameter.

At present there is no easy way to measure the epidermal thickness *in vivo*, therefore the data published by Whitton (Whitton JT, 1973) was used. It is the most comprehensive work on epidermal thicknesses that could be found. Only areas of the skin that are usually exposed to sunlight were selected because that would be the areas most prone to skin cancer. In those areas the published values of the epidermal thickness varies from 39 μm on the cheek to 85 μm on the back of the hand. For easier comparisons, the epidermal thickness in the model was varied between 40 and 90 μm .

Two skin cancers prevalent in South Africa, squamous cell carcinomas (SCC) and nodular basal cell carcinomas (NBCC), were evaluated. For comparison purposes the tumours were assumed to be embedded 0.2 mm into the skin. Optimisation of the model in terms of the voxel sizes and number of photons launched was done in the original model development in Chapter 3. A laser beam diameter of 1.2 cm was used to simulate a typical laser that may be used in a clinical setting. Such a laser was used in some of the *in vitro* cell work that led to the defining of the laser dose parameters (Karsten AE, 2011), (Maduray K, 2011).

Due to the thin epidermal layer, the evaluation slice thickness was reduced to 0.01 mm. The number of slices in depth (Z-direction) was varied to keep the slice thickness constant. The value of g was constant at 0.8 (using the data from Salomatina (Salomatina E, 2006)). The output power of a typical laser would be around 50 mW. In section 3.5 it was shown that if the input laser power was 1 mW the results can be scaled up to the required input laser power by simple multiplication. The results in section 3.5 clearly indicated that the output values for the model when 10 mW was used are 10 times the values when an input power of 1 mW was used. For simplicity an input power of 1 mW was used which allows for easy ‘upscaling’ when more powerful lasers are required.

Even though absorption coefficients as high as 3.36 mm^{-1} (at $\lambda=676 \text{ nm}$) were measured with the reflectance probe, the values were constrained to 3 mm^{-1} , to be conservative. As reported in section 4.4.1, the values of the absorption coefficient measured with the reflectance probe were higher than previously reported values.

There are numerous reports on blistering occurring as a result of laser hair removal treatments (Adrian RM, 2000), (Lanigan S, 2003), (Battle E, 2004), (Lepselter J, 2004). Laser hair removal is a procedure that has been used for a number of years where the hair follicles are irradiated by a laser to prevent or slow hair growth. For the laser light to reach the follicle it needs to penetrate the epidermis. In this application of red lasers (wavelengths around 630 nm) the effect of epidermal absorption is important (see Figure 2.8 section 2.3.1.3). One of the conclusions of laser hair removal for skin phototypes V-VI is that longer pulses (i.e. longer treatment time) must be used (Lepselter J, 2004). This is in line with the findings of this work (see the paper in section 5.1) that for the treatment dose to stay the same, the irradiation time should be increased rather than increasing the laser power to limit possible side effects e.g. burning or blistering.

Results of the computer model are presented in the paper in the next section. In the model the typical spread in epidermal absorption coefficients of the South African skin phototypes

were used (as measured with the diffuse reflectance probe, section 4.4.1). The major advantage of the computer model was that the extent of the absorption effect could be quantified. Use of the model allows the clinician to compensate for the absorption and establish safe and effective treatment power and times before treatment commences. When comparing treatment time between skin of phototype I and V and keeping the fluence rate constant at 44.2 mW/cm^2 , the treatment time is increased from 235 s (phototype I) to 374 s (phototype V), an increase of more than 50 %.

The paper based on epidermal influence on light penetration has been submitted to the journal: Lasers in Medical Science. The paper is included in section 5.1.

5.1 PAPER ON THE EFFECT OF THE EPIDERMIS ON LASER TREATMENT PARAMETERS

The paper on the effect of the epidermis on the treatment parameters is currently under review for publication in Lasers in Medical Science.

As in the earlier papers in this thesis, the format has been altered by changing the Figure and Table numbers to follow numbering in the thesis. The referencing style has also been changed to follow the style of the thesis.

Effect of epidermal absorption on PDT dose calculations

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ABSTRACT

Skin cancer treatments such as Photodynamic Therapy (PDT) rely on light, generally obtained from a laser source, to activate a drug. Penetration of laser light through human skin is highly dependent on the optical properties of skin. The absorption coefficient of the epidermis varies with the amount of melanin in the epidermis (or the skin phototype). The absorption and scattering of the light in the outer skin layers determine the fluence rate of the light reaching the intended treatment site. For effective treatment the losses due to the scattering and absorption must be taken into account. A two layer skin model with an embedded tumour was developed in the ASAP raytracing software environment. The spread in absorption coefficients for the typical South African skin phototypes (Caucasian, African and southern Asian descent) were used as input to the computer model to determine the fluence rate reaching the tumour at a depth of 200 μm . When the epidermal thickness is taken into account for various sun exposed skin areas, the treatment time (for a fixed laser power density) may increase from 235 s to as much as 374 s to deliver the same dose to the tumour.

Keywords: epidermal absorption modelling, South African skin phototypes, light transmission modelling, photodynamic therapy

INTRODUCTION

The effectiveness of lasers in photodynamic treatment (PDT) of cancerous tumours has been described widely (Sharman WM, 1999), (Brown SB, 2004), (Wilson BC, 2008), (Sekkat N, 2012). In the initial testing phase of a potential treatment, tests are done in biochemical laboratories with single cell layers in a Petri dish. Adapting the treatment parameters to be used in clinical settings on patients is not a trivial process. Not all tumours are situated on the surface of the skin. In most clinical applications the laser light needs to penetrate through some skin layers before reaching the treatment area or tumour. The optical properties of the skin layers influence the light propagation through the tissue and as such the distribution of the laser fluence rate at any given depth into the tissue.

“Skin cancer (consisting of basal cell carcinomas (BCC), squamous cell carcinomas (SCC) and malignant melanoma) is the most common type of cancer in the South Africa with about 20 000 reported cases each year” (Mqoqi N, 2004). Australia and South Africa have the highest incidence of skin cancer in the world (Cansa, 2001), (Mqoqi N, 2004). “Basal cell carcinoma accounts for more than 90 percent of all skin cancers. Basal cell cancer grows slowly and does not usually spread to other parts of the body. However, if left untreated, it can spread to nearby areas and invade bone and other tissues under the skin. Squamous cell cancer is much less common than BCC. SCC can be more aggressive than BCC and is also more likely to grow deep below the skin and spread to distant parts of the body. When squamous or basal cell skin cancers are found early, there is nearly a 100 percent chance for cure” (National Cancer Institute, 2011).

PDT is one of the newer cancer treatments (Robertson CA, 2009), (Sekkat N, 2012). PDT is a procedure in which a photosensitiser (PS) or drug is administered orally or topically to the patient and after the absorption of the PS in the cells, the tumour is irradiated with a light source (normally a laser) tuned to an absorption peak of the PS in the visible to near infrared region of the light spectrum. The formation of highly reactive singlet oxygen in the cells leads to cell death through apoptosis or necrosis (Kessel D, 1998), (Bouchier-Hayes L, 2005), (Robertson CA, 2009). Due to the short lifetime of the singlet oxygen in biological systems only molecules close to the PS are affected. The half-lifetime of singlet oxygen is less than 40 ns, and the radius of the action of singlet oxygen is in the order of 20 nm (Castano AP, 2004). PDT is a localised treatment, having

less of an adverse effect on the healthy cells not irradiated by the light source. PDT has been approved in a number of countries for selected cancers and drugs (Calzavara-Pinton PG, 2007), (Sekkat N, 2012).

Human skin is considered a turbid medium due to the highly scattering nature of skin for light in the visible and near infrared wavelength regions (the wavelength region of interest for most laser treatments). In skin the multiple scattering and absorption of light attenuates the laser fluence rate. Computer modelling is a technique that can be used to determine the laser fluence rate reaching a pre-determined depth in the skin. Three of the most important optical properties describing the propagation of light through tissue are the absorption (μ_a) and scattering (μ_s) coefficients as well as the anisotropy or direction of scatter (g). In order to reduce the complexity, the last two parameters are usually combined as the reduced scattering coefficient μ'_s , ($\mu'_s = (1 - g)\mu_s$) (Pfefer TJ, 2003), (Fabrizio M, 2010). In most of the systems used to measure the optical properties of tissue, values of μ'_s are measured and not μ_s (Dam JS, 2000(a)), (Zonios G, 2006), (Johns M, 2005).

Skin can be modelled as a layered structure. With the optical properties of each layer known, laser light can be traced through the skin to determine the losses due to the absorption and scattering of the light as well as the spreading of the light.

The epidermis of the skin contains the melanocytes that are responsible for the melanin production which in turn is responsible for the skin phototype or skin colour. A higher absorption coefficient is associated with the darker skin phototypes. The thickness of the epidermal layer is a function of the position on the human body (Whitton JT, 1973).

A computer model was developed in ASAP, a ray tracing software package from Breault Research, to predict the laser fluence rate reaching a pre-determined depth into skin. Here the model is applied to the prediction of optimal treatment parameters in PDT applications for skin cancer. In general, the first screening tests for PDT applications are done in *in vitro* cell samples (cancerous and normal, healthy skin cell, e.g. fibroblast cells). In this application the cells are irradiated directly with the laser without any outer cell layers on top of the sample (Robertson CA, 2009), (Maduray K, 2011), (Nombona N, 2011) as would be the situation in the *in vivo* treatment applications. Computer modelling can be an effective tool to bridge the step between *in vitro* laboratory tests and clinical tests (both animal and human). It can also be used to illustrate the effect of the light absorption in the epidermis due to the different skin phototypes.

The work presented here was done with a computer model which is able to determine the effect of both the epidermal thickness and the absorption coefficient of the epidermis on the light transmitted 100 μm and 200 μm into a skin. The model consisted of an epidermal and a dermal layer with a tumour embedded in the dermis. The expected range of epidermal absorption coefficients present in the South African population was used.

MATERIALS AND METHODS

Monte Carlo ray tracing programs are efficient in predicting the behaviour of laser light transmission through various media, including human tissue (Jacques S, 2008(b)), (Breault Research, 2006). ASAP is a Monte Carlo ray tracing software package that allows the user to follow multiple rays through a pre-defined geometry (Breault Research, 2006). Each ray is traced until it is absorbed in the model or leaves the boundaries of the model.

In ASAP the anisotropy is substituted into the Henyey-Greenstein equation (Henyey L, 1941) to determine the scattered light distribution as a function of the angle (θ) (Michel B, 2005), (Stevenson MA, 2009):

$$p(\theta) = \frac{1}{4\pi} \frac{1 - g^2}{[1 + g^2 - 2g \cos(\theta)]^{3/2}} \quad (5.1)$$

where the anisotropy, g , is the average directional cosine of the scattered light with a value between -1 and 1.

The ASAP volumetric scatter model is based on the angular distribution and the radiative transport equation (Ishimaru A, 1989) and can be written as (Michel B, 2005), (Stevenson MA, 2009):

$$\frac{dI(r, \omega)}{ds} = -(\mu_a + \mu_s)I(r, \omega) + \frac{\mu_s}{4\pi} \int_{4\pi} p(\omega, \omega')I(r, \omega')d\omega' \quad (5.2)$$

with

$I(r, \omega)$ = radiance at position r directed towards the solid angle ω

$\frac{dI}{ds}$ = directional derivative of I

$p(\omega, \omega')$ = phase function describing the angular distribution of the scattered light

In the model the skin was described as a multilayered system. For each layer the following optical properties need to be specified.

- Geometrical description of the layer
- Absorption coefficient (μ_a)
- Scattering coefficient (μ_s)
- Refractive index (n)
- Anisotropy (g)

In skin the optical properties within layers may vary, but for purposes of this model the assumption was made that optical properties are homogenous within each layer. This model has been tested previously by comparing the transmission through the model with that measured on both liquid and solid skin representing phantoms on an integrating sphere (IS) system (Karsten AE, 2012(a)).

Data regarding the optical properties of human skin are widely published (Cheong WF, 1990), (Tuchin V, 2007), with the most comprehensive reference the book by Tuchin (Tuchin V, 2007). Obtaining optical properties from the literature is not an easy process due to the variance in the published data in terms of skin site, measurement technique used and sample handling for which information is reported. Most of the published measurements were performed on ex vivo skin samples (Tuchin V, 2007). The absorption coefficient, μ_a , in the epidermis is an important parameter that depends on the melanin concentration in the epidermis. In the published literature the variance in μ_a , due to changes in the epidermal melanin concentration, is not readily available. For this work, values measured with a diffuse reflectance probe on 30 individuals in South Africa (volunteers of European, African and Indian subcontinent descent) were used as the upper and lower limits for the μ_a values (Karsten A, 2012(c)).

Salomatina (Salomatina E, 2006) used an IS system to measure the optical properties of the epidermis, dermis and non-melanoma cancers (nodular basal cell carcinomas (NBCC), infiltrative basal cell carcinomas (IBCC) and invasive SCC) separately. For consistency all the optical properties used in the model are from Salomatina (Salomatina E, 2006), except for the absorption coefficient of the epidermis. Both SCC and NBCC were used in the model.

A two-layer skin model (see Figure 5.1(a)) was described consisting of an epidermal and a dermal layer with a tumour embedded in the dermis. In the skin model the skin layers are described as planar surfaces however in actual skin the upper part of the dermis is not planar. To evaluate the influence of an un-even epidermal/dermal interface, a 'wavy' skin surface was constructed as a comparison to the planar interface. An Optical Coherence Tomography system (OCT 1300SS from Thorlabs) was used to do non-invasive measurements on the arms a few volunteers in order to establish a typical profile of the epidermal-dermal interface. In the model a wavy interface was derived from a sawtooth profile that is rounded on the top resulting in a profile as shown in Figure 5.1(b). A circular, Gaussian laser source (output power of 50 mW and beam diameter 1.2 cm) at a wavelength of 676 nm was defined and traced through the model. Two evaluation detectors were put in place. A semi-sphere back-reflection detector was placed behind the laser source to collect all the light reflected from the model (both direct reflections and diffused reflections). A flat, circular transmission detector was placed 0.2 mm behind the last surface of the dermis and used to collect all the light that was transmitted through the model. These detectors, combined with the absorption data in the model were used to ensure that all the rays (total input power) can be accounted for. The dimensions and optical properties of the different layers are listed in Table 5.1. The value of the anisotropy (g) was kept constant at 0.8 for all the layers and the refractive index (n) for the epidermis was 1.5 and for the dermis and tumour 1.4 (Salomatina E, 2006).

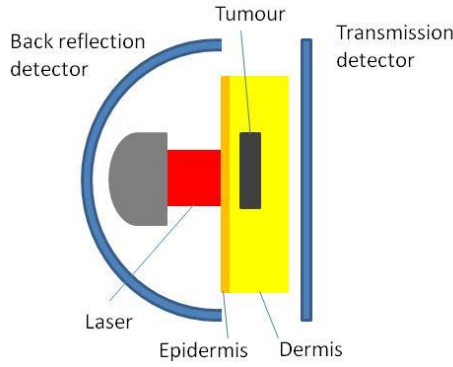


Figure 5.1(a): Schematic of the computer model with the detectors and the different layers.

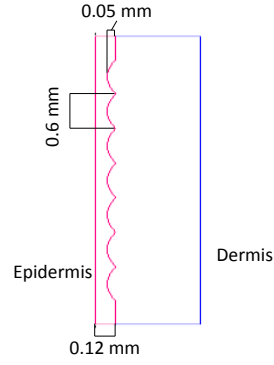


Figure 5.1(b): Structure of the un-even epidermis-dermis interface.

Table 5.1: Geometrical dimensions for the planar surfaces and optical properties of the different layers in the skin model.

Layer	Width (mm)	Start position (mm)	Thickness (mm)	μ_a (mm^{-1})	μ_s (mm^{-1})	μ_s' (mm^{-1})
Epidermis	40	0	0.04-0.09	0.002-3	22.4	4.48
Dermis	40	0.04-0.09	3	0.15	13.9	2.78
Tumour (SCC)	10	0.2	2	0.11	8.55	1.71
Tumour (NBCC)	10	0.2	2	0.09	10.35	2.07

The thickness of the epidermis depends on the skin site. For this work skin sites that are commonly exposed to sunlight were chosen. The epidermal thickness (d_{epi}) varies from 39 μm on the cheek to 85 μm on the back of the hand (Whitton JT, 1973). At other skin sites the epidermis may be much thicker (up to 369 μm at the fingertip (Whitton JT, 1973)). Due to the influence of the absorption coefficient on the light that penetrates into the skin, different epidermal thicknesses were modelled. For the current model d_{epi} was varied between 40 and 90 μm . The values of μ_a were varied between 0.002 and 3.0 mm^{-1} , according to the measurements reported in (Karsten A, 2012(c)).

The wavy interface was only evaluated on the thicker epidermis of 90 μm . The effect of having the interface at a depth of 0.09 mm and 0.12 mm was evaluated as well as changing the height of the wave from 0.025 mm to 0.05 mm. Evaluations were only done for μ_a values of 0.1, 1, 2 and 3 mm^{-1} . The length of a wave was kept to 0.6 mm as indicated in Figure 5.1(b).

In the model the laser beam consists of 3.1 million rays which were traced separately through the model (Karsten AE, 2012(a)). The model is divided into evaluation voxels of 0.2 mm x 0.2 mm x 0.01 mm (X, Y, Z). The accumulated absorbance and fluence rate up to a depth of 100 μm (the depth just after the thickest epidermal layer) were evaluated as well as the light reaching the two evaluation detectors. The optical properties of the two tumours do not differ dramatically, but the difference has a slight influence on the laser fluence rate in the skin.

RESULTS AND DISCUSSIONS

In the models the percentage of the light reflected and detected by the back reflection detector was monitored and reported in Table 5.2. Very good correlation was found between the planar model and the model where the epidermal wave interface started at a depth of 0.12 mm (epidermal thickness of 0.12 mm) with a wave amplitude of 0.05 mm. The results vindicate the use of a planar epidermal/dermal interface as the integrated effect of a wavy interface can be correlated with an equivalent effect from a planar interface at a slightly different depth. The equivalent depth of the planar interface can be approximated by the average of the troughs and peaks of the wavy interface. The rest of the evaluations were done with a planar epidermal/dermal interface.

Table 5.2: % Light reflected from the model for the different wave interface parameters described above.

μ_a (mm^{-1})	Epidermal thickness 0.09 mm		Epidermal thickness 0.12 mm		Planar model (epidermal thickness 0.09 mm)
	Wave height 0.05 mm	Wave height 0.025 mm	Wave height 0.05 mm	Wave height 0.025 mm	
0.1	37	38	38	38	38
1	30	29	28	27	27
2	25	23	21	20	21
3	21	19	17	16	17

For ease of comparison, all the evaluations are reported at a depth of 100 μm into the model (the upper part of the dermis) even though the epidermal thickness ranged from 40 to 90 μm .

In Figure 5.2 the fraction of the light that is absorbed in the first 100 μm of the model is presented as a function of μ_a for the different epidermal thicknesses for both the NBCC and SCC cells. The optical properties of the dermis and the scattering coefficient for the epidermis were kept the same for the different μ_a values even though there is evidence that the dermal μ_s values increase in higher absorbing skins, (Tseng S, 2009), (Karsten A, 2012(c)).

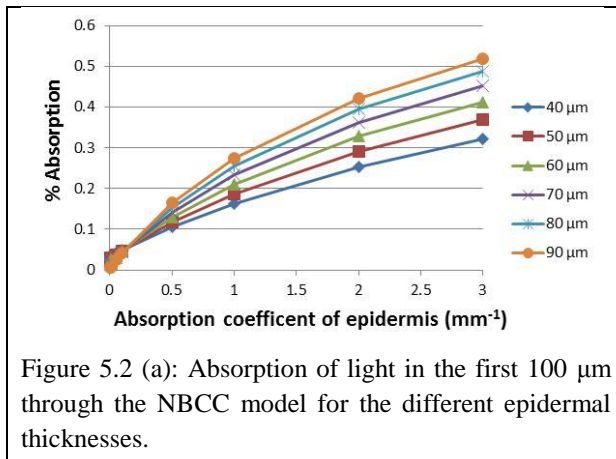


Figure 5.2 (a): Absorption of light in the first 100 μm through the NBCC model for the different epidermal thicknesses.

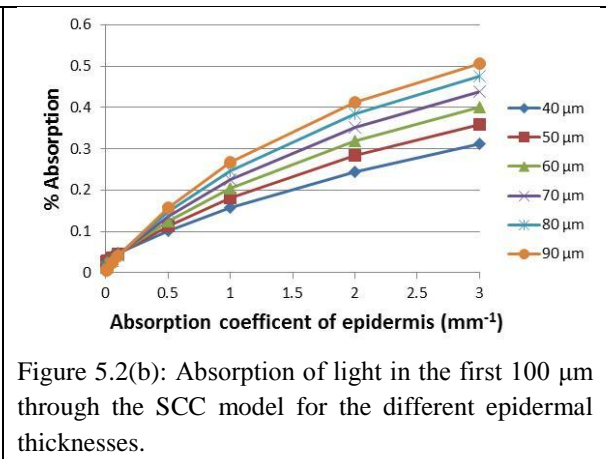


Figure 5.2(b): Absorption of light in the first 100 μm through the SCC model for the different epidermal thicknesses.

The trends for the two different tumours are very similar. The differences are due to the difference in the optical properties of the tumour. For the highest μ_a , up to 50% of the incident light is absorbed in the thick epidermis and just over 30% for the thinnest epidermis. For $\mu_a = 3 \text{ mm}^{-1}$, the absorption is one to two orders of magnitude higher than at the very low μ_a values.

The fluence rate reaching 100 μm into the skin for the two different tumours are presented in Figure 5.3.

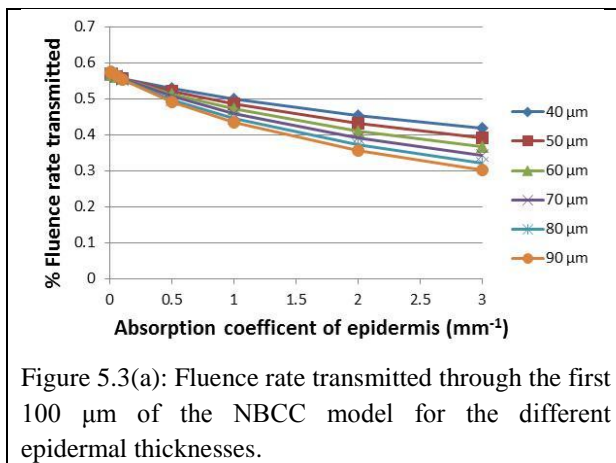


Figure 5.3(a): Fluence rate transmitted through the first 100 μm of the NBCC model for the different epidermal thicknesses.

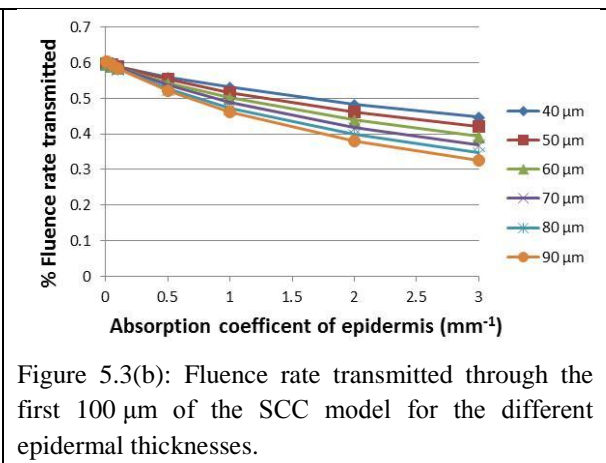
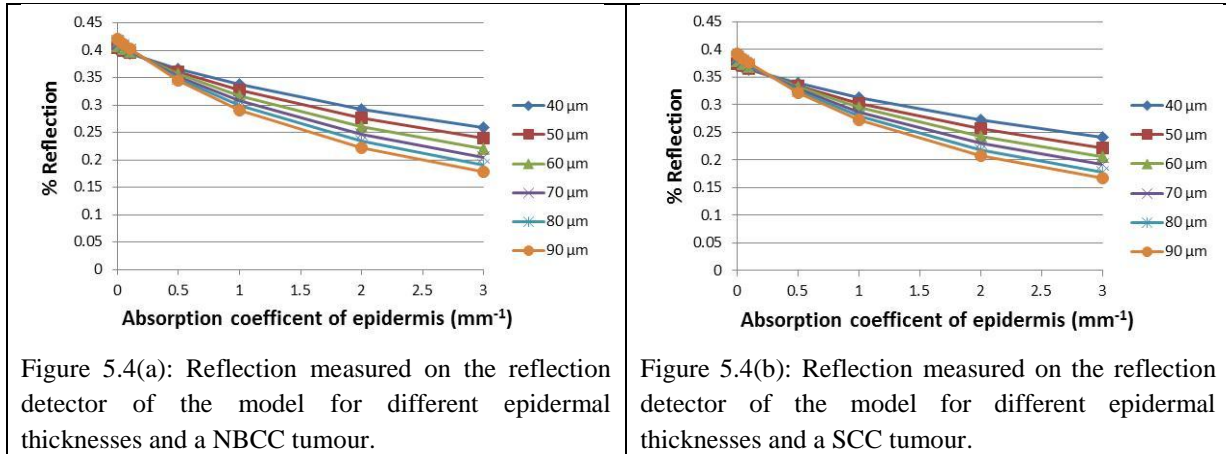


Figure 5.3(b): Fluence rate transmitted through the first 100 μm of the SCC model for the different epidermal thicknesses.

As expected, the transmitted fluence rate values decrease with an increase in the absorption coefficient and with an increase in epidermal thickness. Once again the effect is similar for both tumours.

In the model all the reflected light (both direct reflection and diffuse reflection) is detected on the back reflection detector behind the light source (Figure 5.1(a)). The measured reflection decreases with an increase in the absorption coefficient (Figure 5.4 (a,b)). The decrease in reflection can be ascribed to the fact that an increase in absorption coefficient reduces the amount of light that can be reflected and is therefore the expected behaviour.



Even though the tumour properties have an influence on the fluence rate just before the tumour, the major contributions are attributable to the epidermal thickness and absorption coefficient.

Clinical applications of the model

For the application of the model in a clinical setting, the fluence rate reaching the tumour site is important. The treatment light dose is normally specified in J/cm² which allows optimising of the contribution of the laser power (normally a continuous wave laser or light source) as well as the treatment time. Apart from the absorption taking place in the skin, the light distribution also changes from the original beam profile due to the scattering in the skin, leading to a larger beam diameter.

In this model the initial laser beam diameter was 1.2 cm and the power 50 mW resulting in a fluence rate of 44.2 mW/cm². A laser treatment dose of 4.5 J/cm² at a wavelength of 676 nm is a typical parameter established for effectively killing SCC with Photosense® (a commercial PS) in a research laboratory setting in a Petri dish. (Karsten AE, 2011), (Maduray K, 2011). This implies that the cells need to be illuminated for 102 s with the laser. The fraction of the light that is absorbed in the outer skin layers is an important parameter and must be taken into account when calculating the laser dose delivered onto the skin. In the model, the beam spread to about 1.3 cm at a depth of 200 μm with a reduced fluence rate of 38 mW/cm². In Table 5.3 the fraction of the initial power (mW) at a depth of 100 μm is listed for the average over all the epidermal thicknesses used in this work to illustrate the effect of the absorption coefficient on the transmitted power.

Table 5.3: Fraction of power reaching 100 μm into the skin, just entering the dermis for the SCC tumour.

$\mu_a(\text{mm}^{-1})$	0.002	0.005	0.01	0.05	0.10	0.50	1.0	2.0	3.0
Power fraction transmitted	0.60	0.60	0.60	0.60	0.59	0.54	0.49	0.43	0.38

The fraction of the power reaching the tumour at a depth of 200 μm into the model is listed in Table 5.4 for the average over all the epidermal thicknesses. The tumour depth used is an assumption for illustrative purposes. The tumour position can be moved as required by the application.

Table 5.4: Fraction of power reaching the SCC tumour at a depth of 200 μm into the skin and the resulting treatment time to deliver a light dose of 4.5 J/cm^2 onto the tumour. The laser beam diameter is 1.3 cm at the tumour depth.

μ_a (mm^{-1})	0.002	0.005	0.01	0.05	0.10	0.50	1.0	2.0	3.0
Power fraction transmitted	0.51	0.51	0.51	0.51	0.50	0.46	0.42	0.36	0.32
Treatment time (s)	235	235	235	235	239	260	285	332	374

For the lighter skins (low μ_a values) typically up to skin phototype III, the transmitted power is nearly constant (about 50% of the initial power). In the darker skin (higher μ_a values) much less power reaches the tumour, only about 30% of the initial fluence rate. This significantly affects the optimal treatment parameters for the different skin phototypes and may require adjustment in treatment time for the darker skins (up to 50% longer).

Both the epidermal thickness and μ_a determine the fluence rate reaching the tumour. In a clinical setting the absorption coefficient can be measured non-invasively before the treatment commences. A diffuse reflectance probe (Zonios G, 2006), (Karsten A, 2012(c)) can be used for these measurements. Specific procedures to maximise the accuracy of the probe are still under investigation. The μ_a for most of the lighter skin phototypes (I-III on the Fitzpatrick scale) is below 0.05 mm^{-1} .

The non-invasive measurement of the epidermal thickness is more problematic and does not fall within the scope of this paper. Data on the epidermal thickness for different skin sites are reported by Whitton (Whitton JT, 1973).

CONCLUSIONS

Both the thickness of the epidermis and the absorption coefficient of the epidermis are important parameters in PDT treatment planning for embedded tumours. An increase in the epidermal thickness results in increased absorption of light in the epidermis. For the absorption coefficients evaluated, the ratio in the absorption between the thinner (40 μm) and thicker (90 μm) epidermal layers stays nearly constant. For the parameters used in this work, the effect of the absorption coefficient in the epidermis (due to the skin phototype) is more important than the effect of the epidermal thickness. The analysis for this work was aimed at typical sun exposed areas of the skin where the incidence of skin cancer is more likely. Other areas of the skin may have thicker epidermal layers which will affect the results. In future work the effect of a non-planar epidermal surface should be investigated as this may potentially have a larger influence on the backscattered light from the epidermis due to the higher differences between the index of refraction for the two media.

In most PDT applications there is a minimum fluence rate required to activate the PS and a maximum beyond which healthy cells will be adversely affected. Within this range, the power of the laser can be adjusted to compensate for light absorbed before reaching the tumour. According to the ANSI laser safety standard (Laser Institute of America, 2007), the safe power density for skin at 676 nm is 200 mW/cm^2 . When nearing this skin damage threshold, the safer option will be to adjust the irradiation time to result in the required dose instead of increasing the laser power. This irradiation time, for the data used in this work, needs to be increased from 235 s for the lightest skin to 374 s for the darkest skin.

The increase in absorption of laser light in the visible and near infrared wavelengths for darker skins as well as blistering has been reported before (Alaluf S, 2002(a)), (Lanigan S, 2003), (Battle E, 2004). This is in agreement with the result presented here where darker skins (higher μ_a values) absorb more of the laser light. The major advantage of this computer model is that the extent of the absorption effect can be quantified and it is consequently possible to compensate for the absorption and establish safe and effective treatment times before treatment commences.

A number of other laser treatments (e.g. hair removal, skin rejuvenation) are typically developed in countries with a more homogenous ethnic base than in South Africa. The absorption coefficients used in the model are typical of that present in the South African population and as such are applicable for the range of South African skin phototypes (inclusive of individuals from Caucasian, African and southern Asian descent). The model can be applied to compensate for different skin types in therapeutic as well as other procedures.

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5.2 VALUE OF THE EPIDERMAL ABSORPTION WORK

In this chapter the range of values for the epidermal absorption coefficient obtained by measurements (Chapter 4) were combined with the computer model (Chapter 3) to illustrate the effect of epidermal absorption through the application of PDT treatment of a cancerous tumour. The principles can however be applied to any skin related laser treatment. In laser treatment planning both the thickness and the absorption coefficient of the epidermis are important parameters that must be taken into account. Darker skin phototypes may require up to 50% longer treatment time (374 s) than lighter skin phototypes (235 s) to deliver the same dose 200 μm into the skin.

For individualised treatment, the ideal situation would be to do a diffuse reflectance measurement on the patient before beginning treatment to determine the absorption coefficient for the treatment location. This value can then be used to adjust either the treatment time or laser power or both, depending on the application.

In the next chapter the objectives set out in the beginning will be evaluated in the context of the results achieved in this thesis.

CHAPTER 6: CONCLUSIONS AND WAY FORWARD

This chapter provides a summary of the important findings of the thesis followed by a comparison between the original objectives and the results of the thesis. The limitations and areas for further research will be discussed as well as the relevance of the work to the broader community.

6.1 BRIEF SUMMARY OF THE MAJOR FINDINGS AND CONTRIBUTIONS OF THE WORK IN THE THESIS

A layered structure computer model to predict the laser fluence rate inside skin was *developed, verified and validated* with optical measurements on skin simulating phantoms (Chapter 3). Optical properties from literature may be used, but the absorption coefficients expected for the different skin phototypes were not available in the open literature.

The diffuse reflectance probe (DRP) system together with the software algorithms developed was able to provide values for the expected range of absorption coefficients for the skin phototypes present in the South African population (Chapter 4). Initial data analysis indicated that the melanin contribution in the algorithm should be separated into the two major melanin types (eumelanin and pheomelanin). The original work on which the DRP measurements was based did not make this distinction and only used the dominant eumelanin, which is generally appropriate for lighter skin phototypes.

Implementing the model with the measured absorption coefficients indicated (section 5.1) the effect of the different epidermal absorption values, as well as the effect of scattering in the outer skin layers. The fluence rate decreases by at least 50% in the first 0.2 mm into the skin due to scattering, irrespective of the skin phototype. For the more absorbing skin phototypes there is a further *loss of about 20% due to epidermal absorption*. With this information available, the laser treatment parameters may be adjusted to compensate, either by increasing the initial fluence rate (if the fluence rate is below the damage threshold and the laser power can be increased) or by increasing the treatment time.

6.2 OBJECTIVES SET OUT AND THEIR ACHIEVEMENT

The objectives set in the beginning of this thesis and how well they were accomplished is described in this section:

6.2.1 Objective 1: To develop a computer model that can predict the laser fluence rate some distance into skin.

This was achieved through the following:

- A layered structure skin computer model was developed in ASAP software. (Chapters 3 and 5)
- The computer model has been successfully validated with optical measurements on skin simulating phantoms. (Chapter 3)
- The model has been applied for the determination of fluence rates inside the skin for different skin phototypes. (Chapter 5)

6.2.2 Objective 2: To develop a non-invasive optical measurement technique to determine the epidermal absorption coefficient of an individual.

This was achieved through the following:

- Using a diffuse reflectance probe system (based on commercially available components), software algorithms were developed to extract the absorption coefficient of the epidermis (or outer skin layers) non-invasively for different skin phototypes. The results established that the principle can be applied, but the study needs to be repeated on a much larger population sample before the system may be usable in a clinical setting. (Chapter 4)
- The extracted data gave the first indication of the range of the epidermal absorption coefficients to be expected across the South African population. (Chapter 4)
- The contributions of eumelanin and pheomelanin were explicitly separated in the data extraction algorithm. This was a requirement for dealing with the results of individuals from Southern Asian descent in the South African population. The concept has been reported in section 4.4.1 in the paper “*Diffuse reflectance spectroscopy as a tool to measure the absorption coefficient in skin: South African skin phototypes*” (Karsten A, 2012(c)).

6.2.3 Objective 3: To determine the effect of skin phototype on the absorption of laser light in the epidermis.

This was achieved through the following:

- The fluence rate loss through the epidermis was quantified using the computer model for the various South African skin phototypes. The results

indicated (as expected) that the darker skin phototypes absorb more of the laser light. For the PDT application used in this work, the darker skin phototypes absorbed 20% more light than the lighter skin phototypes.

- Once effective and safe laser skin treatment parameters have been established for a specific procedure and a specific skin phototype, the computer model may be used to predict the parameters for other skin phototypes. This ‘scaling’ effect (up or down) is wavelength dependant and the model should be applied at the specific treatment wavelength.

6.3 LIMITATIONS OF THE WORK

- The planar skin model used did not take the possible effect of a wavy epidermal outer surface into account. Tests on the effect of a wavy epidermal/dermal interface showed that the results were similar when compared with the corresponding planar surface (mainly due to the small difference in the refractive index between the two layers). The effect may be larger when the wavy surface is the air-epidermis boundary (refractive index 1 and 1.5 respectively). This was not investigated in the present work, because the focus was on the effect of the epidermal absorption and a simpler model was chosen.
- The data collected on the range of epidermal absorption coefficients for the South African population during the diffuse reflectance probe measurements was insightful and sufficient for the original objective of that part of the work. In order to use the data with confidence to determine an individual’s epidermal absorption coefficient, the work needs to be expanded to a much larger sample of the population. To enhance accuracy more than three measurements should be done at each measurement point.
- The probe system used in the measurements did not allow for probing different depths into the skin. A system with multiple collecting fibres located at different distances from the emitting fibre may allow for probing at different depths or skin layers.

6.4 FURTHER INVESTIGATIONS IDENTIFIED

The major issues identified in this work that need further investigation:

- The diffuse reflectance probe measurements should be refined on a much larger sample of the South African population in order to apply the technique with confidence in a clinical setting.
- The diffuse reflectance probe and computer models are only valid for ‘near dermal’ applications. For applications deeper into the skin, the effect of blood vessels should be added to the model. This may constitute the next phase of the work.
- A diffuse reflectance probe system with multiple collecting fibres should be developed to test if it will be suitable for probing deeper skin layers or at other depths.
- The exact relationship between the melanin concentration extracted chemically from skin biopsies and the absorption coefficient deduced by optical means (diffuse reflectance probe) has not been clarified. This needs further investigation as well as how these parameters relate to the diffuse reflectance probe measurements.

6.5 CONTRIBUTIONS OF THE WORK TO THE SCIENTIFIC COMMUNITY AND THE WIDER PUBLIC

6.5.1 Contribution to the scientific community

- The computer model is an uncomplicated planar model. The use of a wavy interface between the epidermis and dermis did not change the fluence rate data significantly and this shows that a planar model is sufficient for this application.
- The model gives a good indication of the expected fluence rate at the required depth into the skin.
- The diffuse reflectance probe measurements and analysis led to the conclusion that both eumelanin and pheomelanin contributions should be accounted for in the data extraction equations. It has a more pronounced influence on people from Asian (and potentially some African) descent, but does not influence the lighter and darker skin phototypes significantly.

- The *in vivo* diffuse reflectance measurements gave the first results of the expected range of the epidermal absorption coefficient for the South African population.

6.5.2 Contribution to the wider public

- The process to establish a new laser treatment technique is a lengthy one. One of the initial steps is to determine the required laser parameters (amongst other parameters) in a biochemistry laboratory on the appropriate cells in a petri dish (*in vitro*). Once success is achieved, one of the next steps is to translate this information to *in vivo* testing. Due to the scattering and absorption of the laser light through the tissue, the laboratory parameters cannot be used unaltered in the initial clinical trials. The computer model developed here has the potential to be used in bridging this gap in order to reduce the number of clinical test required in the development process.
- The non-invasive diffuse reflectance measurements, performed on a patient before treatment commences, in combination with the computer model may ensure more accurate and individualised treatment parameters.

6.6 CONCLUSIONS

This thesis documents the development of a layered planar computer model to predict laser fluence rate at some depth into skin. The computer model was validated and calibrated against optical measurements on skin simulating phantoms. It was shown that a planar epidermal/dermal interface is adequate for simulation purposes.

The epidermal absorption coefficients of a small sample of the South African population were measured with a diffuse reflectance probe system. These measurements were the first to be done to determine the expected range of skin phototypes of the South African population.

Given the positive results, the computer model was used to evaluate the effect of the epidermal absorption coefficient (a parameter dictated by an individual's skin phototype) to predict the adjustment in laser treatment times for different absorption coefficients. Application of the computer model to photodynamic treatment of skin cancer indicated that treatment times need to be adjusted (by as much as 50%) when comparing very fair skin phototypes with very dark skin phototypes.