Robustness and accuracy of the calibration model for the determination of the optical properties of chicken skin

A Singh*, A E Karsten, J S Dam
*CSIR National Laser Centre, Biophotonics Group, PO Box 395, Pretoria, 0001, South Africa
Email: ASingh1@csir.co.za

INTRODUCTION

Light propagation in tissue is at the core of redefining conventional medical therapies and diagnostic techniques. Such methods exploit the properties of light in the form of lasers, LED's or white light sources making it imperative that the interaction between the tissue and light is understood. Although the therapeutic effects of light have been known for ages there is now an even greater need to understand the mechanisms of such methods like optically based wound healing and photodynamic therapy (PDT) [1,2]. Excessive deposition of light on tissue surfaces has the potential to damage the tissue and its surroundings. However, knowledge of the optical properties such as the absorption and scattering or reduced scattering coefficients (μ_a, μ_s and μ_s' respectively) as well as the anisotropy factor (g) assists in determining the dosage amounts required for treatment or diagnosis with minimal to no damage to the surrounding tissue. Although some of these properties do exist in literature, the composition of tissue varies, as revealed by this study, and where possible it is preferred that these properties are measured, in situ. For lab scale research, the gold standard for measurement of bulk optical properties of in vitro samples is the Integrating Sphere. As shown in Figure 1, the human skin is a diverse organ consisting of different layers composed of various structures and components. These diverse components cause light incident on the tissue to either be reflected off the surface, scattered within the tissue, absorbed by the tissue or transmitted through as shown in figure 1. Thus by measuring the diffuse reflectance(R) and transmittance(T) as well as the total and collimated transmittance(Tc) all the parameters μ_a, μ_s and μ_s' and g can be determined [3].

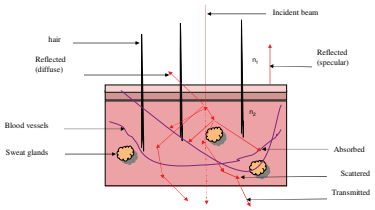

Figure 1: Illustration of the basic phenomena accompanying the interaction of light with skin/tissue

Both μ_a and μ_s' can be extracted from the reflectance and transmission measurements on the integrating sphere setup, together with the calibration model. μ_s and g can be determined from collimated transmission measurements using the Beer-Lambert Law

$$I = I_o e^{-\mu_t \cdot d} \qquad (1)$$

Where $\mu_t = \mu_a + \mu_s$ and d is the thickness of the sample. The anisotropy factor g can then be calculated

using $\mu_s' = \mu_s (1 - g)$ (2)

For the present investigations, the optical properties (μ_a and μ_s') are determined from the measurements using a calibration model and are extracted using the multiple polynomial regression method [4].

MATERIALS AND METHODS

A 7.4 mW He-Ne laser(λ=632.8nm, JDS Uniphase laser) laser was coupled into a multimode fibre(core diameter 62.5 um) using a 10X(numerical aperture(NA) = 0.25) microscope objective and collimated to a beam diameter of ~2mm using a 40X(NA=0.65) microscope objective. Two sample holders with a diameter of 25mm were connected onto the entrance and exit ports of the 8 inch diameter Integrating Sphere (Labsphere). The signal is input to the detector (Ocean Optics USB4000 spectrometer) using a fibre(600 um, Ocean Optics). Reflectance and transmission measurements for each sample were taken in triplicates. A calibration model with known μ_a and μ_s' was created using intralipid (scatter, non-absorbing) and black ink (absorber, non-scattering) solutions.

The first set of the model was in the range $0.545 \le \mu_a \le 5.815$ cm-1 and $11.04 \le \mu_s' \le 55.2$ cm-1. The model was then extended for $0.435 \le \mu_a \le 4.69$ cm-1 and $66.24 \le \mu_s' \le 110.4$ cm-1. Different order polynomials fits were computed onto the measured R and T values. The prediction errors for each polynomial fit in extracting μ_a and μ_s' is shown in Table 1.

Once the calibration model was set up, chicken skin taken off chicken bought from a local grocery store was evaluated. Chicken skin is stated to be optically similar to human skin [5]. Three pieces of the same piece of chicken was measured to illustrate the inhomogenuity of the tissue, the skin was also evaluated as straight from the fridge and hydrated in water for 2 hours. The optical parameters were then extracted from the calibration model using the Newton Raphson method.

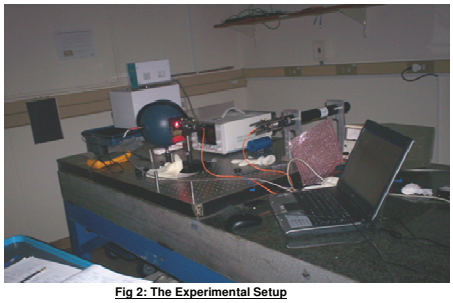

Fig 2: The Experimental Setup

RESULTS AND DISCUSSION

Table 1: The Mean Error of Predictions for the different calibration models created, expressed as a %, using the multiple polynomial regression method (NaN is "not a number").

Model	Parameter	2nd	3rd	4th	5th
1st set inner matrix	μ_a	4.60	1.72	0.8295	2.95
1st set inner matrix	μ_s'	3.46	2.08	1.36	>100
Comb. set inner matrix	μ_a	5.01	2.81	1.792	NaN
Comb. set inner matrix	μ_s'	6.68	3.86	1.775	NaN

The prediction results shown in Table 1 indicated that for the first and the combined model, the 3rd and 4th order polynomial appeared to be the most robust and accurate. (Inner matrix means only the non boundary samples of the model were predicted based on the measurements done). Hence, in most evaluations the 3rd and 4th order predictions were used. Figure 2(a) and (b) show the predictions produced by the 3rd order polynomial which was shown to work well even when μ_a and μ_s' of the sample, as in this case, fell close to the boundary of the calibration model. This reflects a robust, efficient and working model.

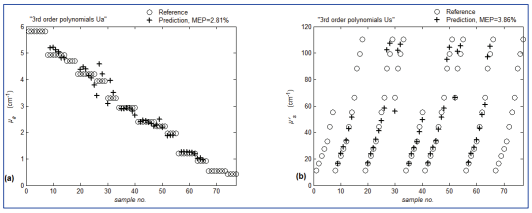

Figure 2(a) 3rd order predictions for μa (b) 3rd order predictions for μs'

The results in table 2 show that there is definitely a difference in the properties obtained for the tissue under different conditions i.e. straight from the fridge and being hydrated and different thicknesses due to the different composition of the two samples.

Table 2: Measured Optical Properties for chicken skin

Type of Sample	Skin upper side		Skin under side	
	$u_a(cm^{-1})$	$\mu_s'(cm^{-1})$	$u_a(cm^{-1})$	$\mu_s'(cm^{-1})$
1.4 mm fridge	0.52±0.0.04	10.85±0.55	0.46±0.0.03	11.0±0.54
1.01mm hydrated	0.44±0.07	14.16±0.8	0.59±0.05	18.05±0.55
1.78mm hydrated	0.2±0.02	20.51±0.07	0.16±0.02	21.42±0.05

These results are also consistent with those of human and pig skin where u_a is higher in the upper part of the skin possibly due to the melanin and is less in the deeper layers. The apparent increase in μ_s' for the thicker part of the skin could be as a result of the inhomogenuity of the sample or due to the presence of some fat. When the properties are measured for the underside of the skin, the absorption is consistently lower than for the skin side measurements except for the 1.01mm slice while μ_s' is consistently higher for all the samples. μ_a and μ_s' for human dermis in literature[6] compares reasonably with these measured values, bearing in mind these are not fresh samples and the literature values vary greatly[7]. A comparative analysis with human skin awaits ethical approval.

CONCLUSIONS

The results illustrated the need to have a well defined and accurate calibration model for *in situ* determination of the optical properties of tissue due to the inhomogenous nature of tissue.

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