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Measurement of an Optical Parameters: Absorption Scattering and Auto-florescence of Skin *in vitro*

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Abstract: In this study the chicken breast skin tissues were illuminated with collimated radiation of 400-700 nm Nd-YAG pumped dye laser and measured skin optical properties for dry and hydrated sample *in vitro*. Total reflectance and transmitted intensities were recorded by which scattering, absorption and anisotropic factors of the sample obtained using double integrating sphere setup. The *in vitro* optical parameters are higher then *in vivo* measurements. Our *in vitro* results are in agreements with other data available in literature. Hydration of skin is found to influence its scattering properties. Dry sample scatter less then hydrated sample. Skin auto-florescence spectra were acquired under different excitation wavelength, it shows difference between normal and malignant tissues.

Key words: Optical imaging, light transmittance, absorption, scattering, auto-florescence, *in vitro*, double integrating sphere

INTRODUCTION

Skin is a turbid medium. It constitutes a protective barrier against physical damage of underlying tissue invasion of hazardous chemical and bacterial substances, through the activity of its sweet glands and blood vessels. It helps to maintain the body at constant temperature. The skin is consist on outer protection layer epidermis an inner layer the dermis and stratum corneum. Upper layer of epidermis, consists of dead cells, the dermis is composed of vascularised fibrous connective tissues the subcutaneous tissue, located underneath the skin, in primarily composed of adipose tissue (fat), skin is exposed to the environment, at which incident radiation is refracted. The refractive index[1] is different from the adjacent structure, which may scatter the penetrating radiation. Scattering and refraction at an irregular interface increase the average path length of the penetrating radiation and thereby need the depth of penetration. The understanding of light transport in tissue is an active and an important research area because of its potential applications in medical diagnostic, therapeutic and surgical procedures[2]. Skin is the region most quantitatively studied. It is representation of other organs and it is the first boundary crossed for many therapeutic uses of light. The effects of optical radiation on human skin ranging in scale from molecular to organ are of considerable attention. To understand various models for radiation transfer through the skin, one has to have knowledge of scattering, absorption, refraction, reflection and transmission of skin. Once such a model is established it become possible to compute from only a few measurements the intensity profile of radiation penetrating the skin. Kubelka-Munk model has been applied several times to skin[3]. The direct measurements of angular distribution of radiation transmitted through skin were performed by Lu *et al.*[4] at high wavelength scattering by stratum corneum and epidermis has measured directly by Du *et al.*[5] he placed skin layer in front of monocharometer, in this way he measured direct transmittance intensity. It is concluded that epidermal scattering is large for irregular refractive and reflective interfaces.

In this study we applied the model *in vitro* for skin tissue coefficients and concentrate on the epidermis, stratum cornium and dermis. The data we obtain for skin optics, will be useful for the study of phenomena such as erythema, carcinogenesis and pigmentation, which are almost certainly influenced by skin transmission.

Many values for the absorption and scattering coefficients determined *in vitro* and *in vivo* have been published[6,7]. The relative reflectance as measured at the

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skin surface decreases much faster for increasing distance then the *in vitro* data suggested[8,9] the optical properties may be changed by freezing, drying[10], heating and by deformation of the sample during measurements[11,12]. Calibrating and measuring procedures may introduce error into the determined values of diffuse reflectance and total transmission deviation in data may occur due to reflectance at tissue-air or glass-air interface, which may be larger then the normal values[13,14], but neglected. In this work we adopted necessary precautions of sample preparation and sample handling. We can improve our results by using numerical methods. Monte Carlo simulation have the advantages of being flexible with respect to geometry, phase function and boundary conditions the disadvantage of Monte Carlo model is large amount of time needed for calculation[15]. We perform the present study to estimate the chicken breast skin optical properties *in vitro* for broad range laser wavelength (400-700 nm).

Auto-fluorescence of skin tissue is an important phenomenon, it can reveal alteration of natural fluorophores composition in pathology and help in precise measurement of tissue optics properties. In this study the auto-fluorescence of skin is studied. To enhance the collection of emitted radiation the skin sample is placed on stainless steel metal surface and illuminated by collimated laser beam.

MATERIALS AND METHODS

The values of absorption coefficient μ_a, scattering coefficients are normally obtained by measuring total reflectance Rtotal and total transmission T_{total}, respectively. The double integrating sphere is consisting of two spheres with the sample placed between them (Fig.1). Both are of same geometry and surface reflectance. The collimated beam of light irradiates the sample in first sphere, called reflectance sphere, a portion of this light will be transmitted through the sample to the second sphere, called transmittance sphere.

Some of the light within the transmittance sphere will irradiate the sample and some of this will be transmitted back into the reflectance sphere. Thus the signal in reflectance sphere will increase. Some of this additional light then re transmitted and the signal in transmittance sphere is increased.

If all the geometric properties of the sphere are known then total reflectance and transmittance can be collected easily. Experimental setup is consisting on double integrating sphere with skin sample, Nd-Yag laser and photodiodes placed on the wall of the sphere and on the exit port of transmittance sphere. The signals from the photodiodes and frequency are feeded into a lock-in amplifier.

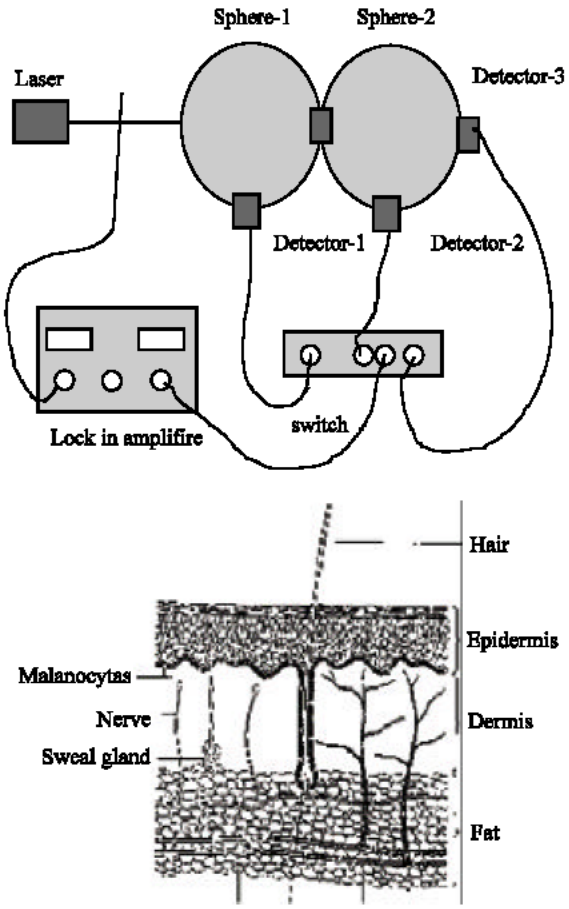

Fig. 1: (a) Experimental setup for measurement of *in vitro* tissue optical parameters (b) Structure of skin

Several studies on the derivation of optical properties of skin samples from measurements with integrating sphere have been published[16]. The chicken breast skin sample was placed on the flat surface of the quartz hemisphere (radius 11 mm), the sample was covered with a circular diphagram of 1mm diameter, due to approximate match of the refractive index of the hemisphere and the sample, the light ray emerging from the skin sample passed through the skin-quartz interface without being refracted, impinged perpendicularly on the optics curved quartz-air interface. By using hemisphere as a sample carrier, we ensured that the angle at witch the light rays emerged remained approximately the same as the angle into which they were scattered in the intact skin. A collimated beam of monochromatic radiation illuminated the sample.

In order to investigate the influence of hydration on the scattering properties of the skin, two samples were hydrated overnight on a saline solution in covered dashes. The data were recorded at several wavelengths for perpendicular incident radiation, the sample was

allowed to dry in room air and intensity is recorded. The transmittance and reflectance data of the sample thickness 8 mm in 400-700 nm wavelength range with the step of 10 nm were obtained using spectrophotometer with integrating sphere to reconstruct the optical parameters, absorption coefficient, scattering coefficients and anisotropic factor. Various approaches have been used Kubelka-Munk theory, Diffusion approximation, Monte Carlo simulation and adding-doubling methods etc, for determination of reliable optical properties and need real geometry of the experiment and angular structure of radiation in the sample. Absorption and scattering coefficients were measured using total reflectance of the sample; anisotropic factor is measured from independent measurements or empirical formula.

RESULTS AND DISCUSSION

The accuracy of determined absorption and scattering coefficients in all the discussed *in vitro* methods depends upon the accuracy of measured total reflectance and total transmation. The results of an experiment are in Table 1.

Measurements of reflectance and transmittance were performed using a double integrating sphere and Nd-YAG laser with detector. The zero transmittance (T_0) was measured by placing chopper between beam and entrance port of the integrating sphere. The 100% transmittance (T_{100}) was determined by placing an empty mass in the sample beam. Sample transmittance and reflectance is measured through detector. The experiment was repeated for hydrated and dry sample of skin, several times. The measurements were made over the 400-700 nm wavelength region. The Rt and Tt was computed using the relation,

$$R_t = (R_s - R_0)/(R^{100} - R_g)$$
$$T_t = (T_s - T_0)/(T_{100} - T_0) \quad (1)$$

The exact description of photon propagation is governed by the radiative transport equation, but it cannot be solved exactly, except for few special cases. Bears' law is a simple model that yields reasonable results in media when absorption dominates scattering. However most biological tissues are highly scattering and Bears' law is not applicable at many wavelengths.

Table 1: Optical properties of chicken breast tissues at 400-700nm from integrating sphere obtained by R_{total} and T_{total}

Author	Calculation method	ua $(mm)^{-1}$	us $(mm)^{-1}$	Anisotropy Factor "g"
Jacques *et al.*	*In vitro*	0.27	2.20	0.871
This work (dry sample)	*In vitro*	0.19	2.38	0.834
This work (hydrated sample)	*In vitro*	0.13	2.48	0.827

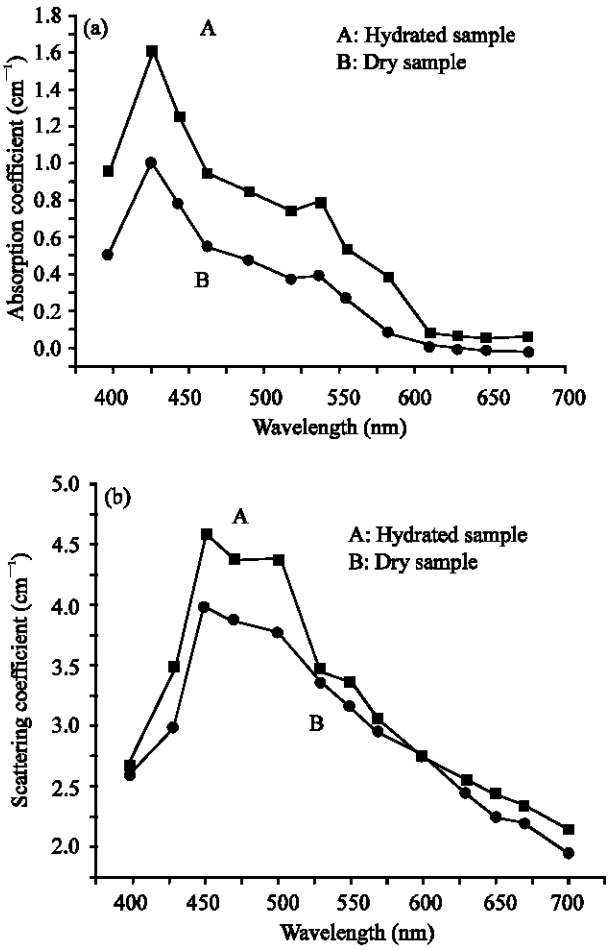

Fig 2: (a)Absorption spectra of chicken breast skin tissue With double integrating sphere (b) Scattering spectra of chicken breast skin tissue, with double integrating sphere

In present experiment the optical parameters, μ_a, μ_s and g are summarized for several wavelength, placing the sample in integrating sphere the absorption and scattering profile for hydrated and dry samples were obtained at several wavelengths, represented in Fig. 2a and b. It is found that as a result of dry skin scattering is reduced. The amount of reduction does not depend strongly on wavelength but on hydration and other factors also, the hydrated sample look turbid and dry as opaque and thin. The general impression from our measurements is of a forward oriented scattering mechanism. Scattering occurs both at surface and throughout the skin medium. The reduce scattering of hydrated and dry sample is maximum at 470 nm (Fig. 2b), as the melanin content show

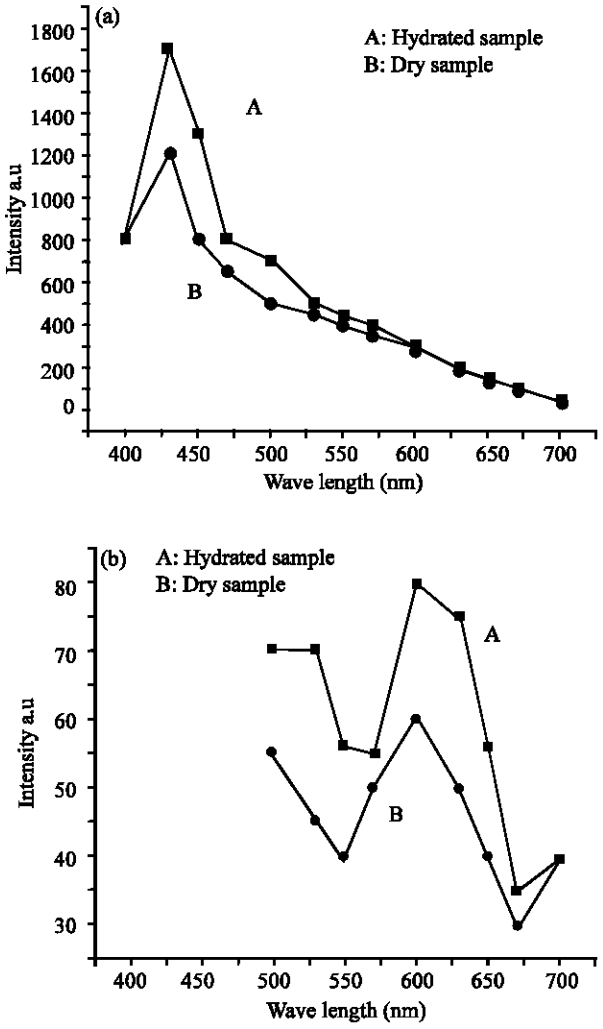

Fig. 3: Auto-fluorescence of chicken breast skin tissue for hydrated and dry sample. (a) Non heated. (b) Heated

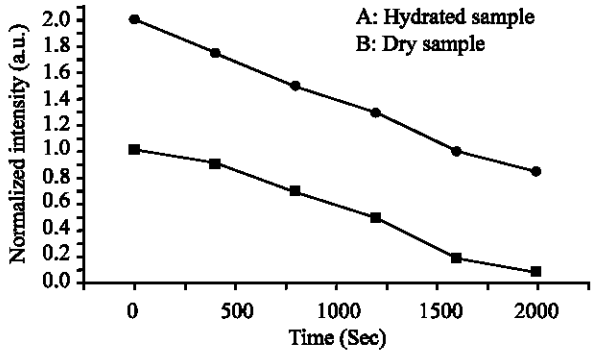

Fig. 4: Transmitted intensity verses time behavior of hydrated and dry sample

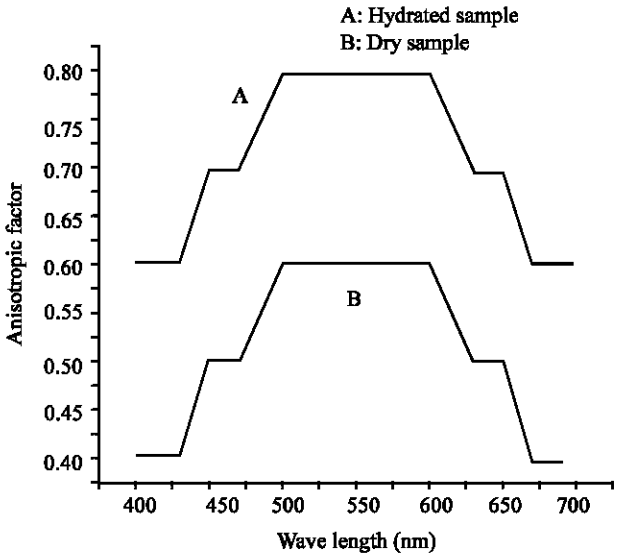

Fig. 5: Anisotropic factor "g" as a function of wavelength

scattering at 470 nm. On comparing our result to Jacques *et al.*[17] it become clear that in our sample scattering is less. The decrease in scattering and absorption, due to surface or volume scattering reduction cannot be separated, volume scattering in Fig 2b, present in hydrated sample disappear in dry sample. When the optics of full thickness skin are being modeled, dermis scattering is the most important, as the penetrating radiation is quickly diffused. It is still just an approximation of the true solution. A more extensive theory would require for sophisticated numerical model for computation, which cannot be performed very accurately because of the large biological variations.

The experimental found values for μ_a and μ_s are influenced slightly by melanin content of epidermis[18].

Therefore small absorption coefficient cannot be determined accurately, as the sum of R_{total} and T_{total} is close to 01. Moreover loss of light within the sample holder may contribute to an increase in the absorption coefficient of tissue. Monte Carlo simulation in good agreement with those measured by Song *et al.*[19].

Absorption measurement evidences a large variation from tissue to tissue of the absorption coefficient value ranging from <02 to 20 cm^{-1}. This fact is easily explainable due to different blood contents, which still remain in the sliced samples. Different samples have variety in absorption coefficients. However integrating sphere in cavity measurements make it possible to estimate reasonably the absorption coefficient of sample with a large scattering coefficient. Scattering measurements shows non-isotropic pattern. The anisotropic g factor value are some what higher then those reported (0.81),

under the hypothesis that cell and there internal structure can be approximated by spherical particles and that the main contribution to scattering is due to the cell envelope. The difference between the measured values of ua and us is due to measuring, calculation techniques and sample handling etc.

The measurements from integrating sphere is not straight forwarded, several studies on accuracy of measuring with integrating sphere shows that errors may be due to that the light which leaves the sample re-enter the tissue sample after reflectance. Measurements of total transmission depend on reflectance properties. One of the reasons of difference in results taken by Jacques *et al*,[17] by our calculations, is that they heated the sample which changes the scattering properties of the tissue. Scattering is almost doubles when the sample is heated. The value of g started to decrease slightly at less then 500 nm and greater then 650 nm (Fig. 5). Reflective index was taken as 1.4 for turbid medium and 1 for surrounding. We consider the rectangular shape of detector[20] different values of μ_a and μ_s can be used to obtain the same intensity ratios at detector.

In Fig. 3 auto-fluorescence data is taken from 400-700 nm excitation wavelength and present the curve average of our several experiments. The data is similar to whole skin *in vitro* auto-florescence and maximum at 420 nm for dry and hydrated sample is maximum at 450 nm wavelength. The results are similar to the results obtained by Kobayashi *et al*.[21]. Florescence responsible for emission in this range may be proteins and NADH.

Although proteins absorption region located at shorter wavelength. NADH considerably presents in upper epidermis layer. In Fig.3 (b) we noted small maximum at 510 nm region. For the whole skin *in vitro* and 442 nm excitation of skin strong peak was observed at 632 nm, this peak is distributed to natural porphyrins (excited band covered short line) in the sample. The spectra of dry skin is taken in dark at room temp, during first 24 h fluorescent intensity decreases by 07% and after four days to 70%, but at shorter wavelength rate of degradation is higher.

The optical properties verses time behavior of the skin tissues is shown in Fig. 4. It concluded that intensity should be measured for skin *in vitro* not later then one day after sampling. The moisture effect should be considered for intensity measurements in Fig. 5 the anisotropic factor g is shown as the function of wavelength and maximum at 500 to 650 nm.

CONCLUSION

The optical property of skin is very important for the light dosimetry. For the exact tissue parameters measurement and spectroscopic study, boundary condition and side way photon loses are very important.

The method of our *in vitro* optical parameter measurement is not suitable to *in vivo*, but it gives important information for skin tissue modeling. The experimental data and the investigations were performed at 400-700 nm (visible and near IR). It is reasonable to assume that the scattering coefficient is slightly dependent on wavelength in the visible range. This is not true for absorption coefficient, since tissue blood content and specific absorbing pigments play the main role in determining tissue characteristic. This fact should be kept in consideration while performing the evaluation of light flux distribution in depth. More efforts, therefore be made to determined more precisely the absorption and scattering coefficients and how they are related to blood flow contents.

Analysis of auto-florescence spectra of skin sample is suggested as a mean for skin status diagnostic and monitoring. The optical property of chicken skin tissue is different for different tissues. The variation in coefficients most likely was due to biological variation, preparations of sample and prolonged freezing time, which leads to cell rapture.

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