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Measurement of Optical Parameters: Absorption Scattering and Autofulorescence of Skin in Vitro

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Abstract.

From an optical point of view the outer most skin layers contain numerous structure by which penetrating radiation may be scattered as well as absorbed. The nature and strength of scattering and absorption may strongly influence the length of penetration.

We illuminated the chicken breast skin tissues 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 setups.

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 autofurescence spectra were acquired under different excitation wavelength, it shows difference between normal and malignant tissues.

Key words: transmittance, reflectance, absorption, scattering, autoflurescence, in vitro, and 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[3-4]. The direct goineometric measurements of angular distribution of radiation transmitted through skin were performed by Heardy et al of high wavelength scattering by stratum corneum and epidermis

has measured directly by Lucas et al,[5] he placed skin layer in front of monocharometer, in this way he measured direct transmitance intensity. Anderson and Parrish 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 durmis. The data we obtain for skin optics will be useful for the study of phenomena such as erythema, carcinogenisis 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 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 as large as 40%[13-14], but neglected. In this work we adopted necessary precaution 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.

Autofluorescence 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 work we studied the auto fluorescence of skin. To enhance the collection of emitted radiation the skin sample is placed on stain steel metal surface and illuminated by collimated laser beam.

Materials and Methods:

The values of absorption coefficient μ_a, scattering coefficient μ_s are normally obtained by measuring total reflectance R_{total} 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 transmitance sphere. The signals from the photodiodes and frequency are feeded into a lock-in amplifier.
Several studies on the derivation of optical properties of skin samples from measurements with integrating sphere have been published[18-19]. Values for R_{total} and T_{total} , "absorption coefficient (μ_a)", "scattering coefficients(μ_s)" and anisotropic factor "g" are calculated by integrating sphere.

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.

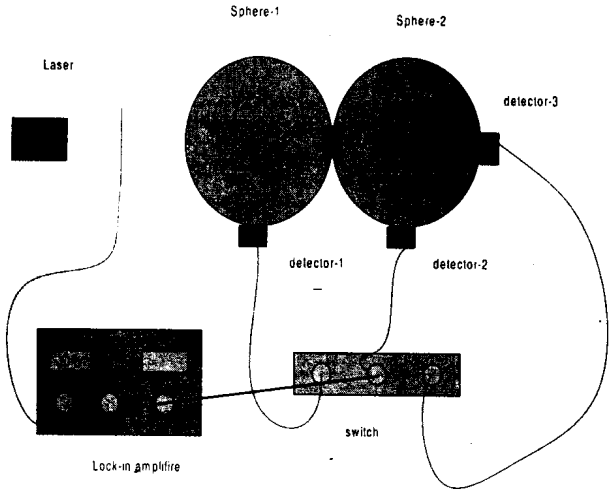


Fig. 1 Experimental setup for measurement of tissue parameters.

Fig.1 (b) Structure of skin

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 8mm in 400-700nm wavelength range with the step of 10nm were obtained using spectrophotometer with integrating sphere to reconstruct the optical parameters, "absorption coefficient", "scattering coefficients" and anisotropic factor "g". 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

Author	Calculation Method	u_a $(mm)^{-1}$	u_s $(mm)^{-1}$	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

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

Measurements of reflectance and transmitance were performed using a double integrating sphere and Nd-YAG laser with detector. Zero transmitance (T_0) was measured by placing chopper between beam and entrance to the integrating sphere. The 100% transmitance (T_{100}) was determined by placing an empty mass in the sample beam. Sample transmitance and reflectance was measured by 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 R_t and T_t was computed using the relation,

$$R_t=(R_s - R_0)/(R_{100}-R_0)$$

$$T_t= (T_s-T_0) / (T_{100}-T_0).$$

The exact description of photon propagation is governed by the radiative transport equation, but it can not 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.

In our 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 fig.2b. 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 470nm(fig.2b), as the melanin content show scattering at 470nm. On comparing our result to Hardy et al, 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 1b, 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 can not be performed very accurately because of the large biological variation.

The experimental found values for u_a and u_s are influenced slightly by melanin content of epidermis[16]. Therefore small absorption coefficient can not 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 absorption coefficient as shown by Pekering et al. Monte Carlo simulation in good agreement with those measured by Prahl et al[17].

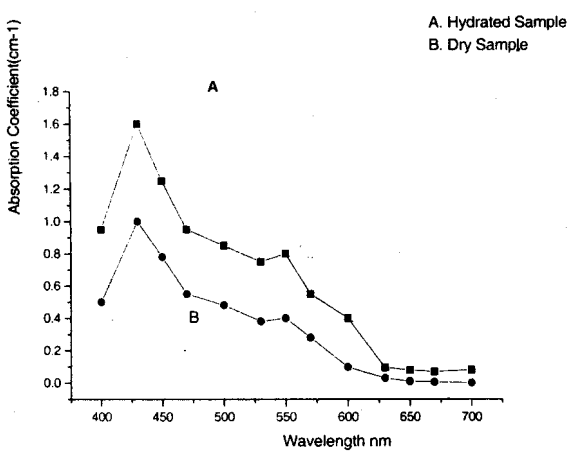


Fig 2a: Absorption spectra of Chicken breast skin tissue With double integrating sphere

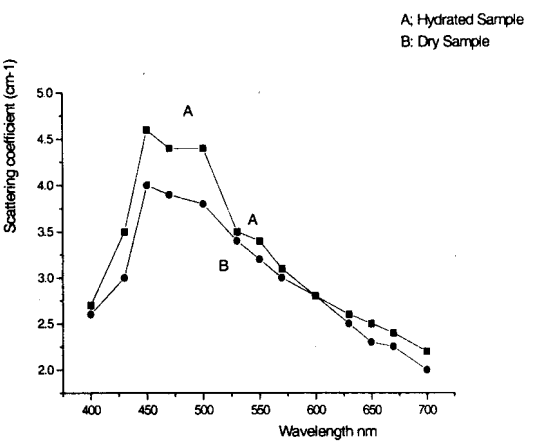


Fig 2b: Scattering spectra of Chicken breast skin tissue, with double integrating sphere

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

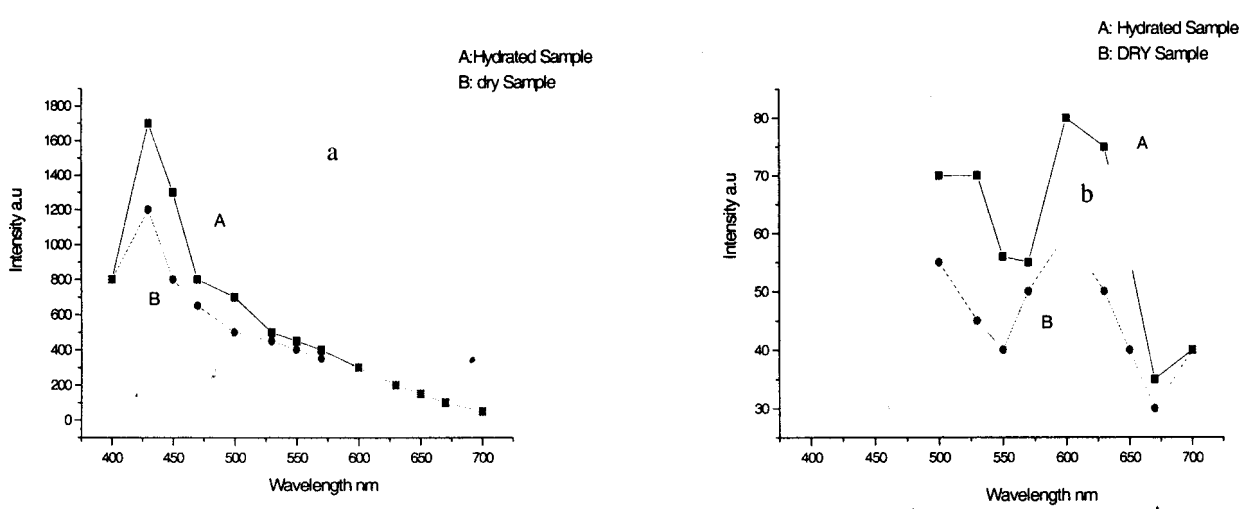
in the sliced samples. Different samples have varity in absorption coefficients. However integrating sphere in cavity measurements[18] make it possible to estimate reasonably the absorption coefficient of sample with a large scattering coefficient. Scattering measurements shows non-isotropic pattern. The anisotropic g factor value are some what higher then those reported (0.81)[19], 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 u_a and u_s is due to measuring, calculation techniques and sample handling etc.

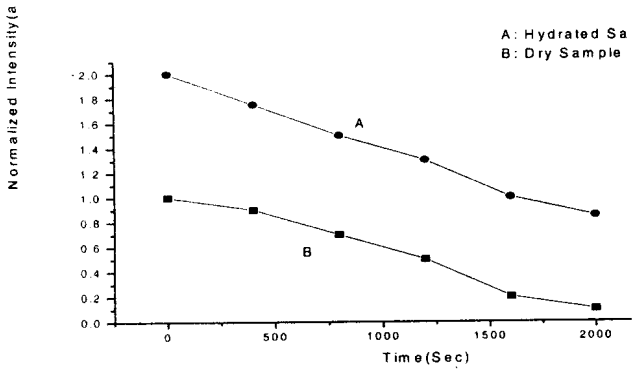
The measurements from integrating sphere is not straight forwarded, several studies on accuracy of measuring with integrating sphere shows that errors may be due to that the light which leaves the sample re-enter the tissue sample after reflectance. Measurements of total transmission depend on reflectance properties. One of the reasons of difference in results taken by Jacques et al and Prahl et al and from 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 heating (fig.6). Reflective index was taken as 1.4 for turbid medium and 1 for surrounding. We consider the rectangular shape of detector, 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 experiment. The data is similar to whole skin in vitro autofluroscence and maximum at 420nm for dry and hydrated sample is maximum at 450nm wavelength. The results are similar to the results obtained by S.Rvtz et al[20].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 we noted small maximum at 510nm region which is comparable to (ref- 20) for the whole skin in vitro and in(ref-24) for 442nm excitation of skin strong peak was observed at 632nm, 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 hrs fluorescent intensity decreases by 07% and after four days to 70%, but at shorter wavelength rate of degradation is higher. The hydrated sample in same conditions shown in fig.4, the intensity rise at 420 nm was 6 time less at 632nm. 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.

Fig: 4 Autofluorescence of Chicken Breast Skin Tissue for Hydrated and Dry Sample. (a) non heated. (b) heated.





Behaviour of Hydrated and Dry Sample.

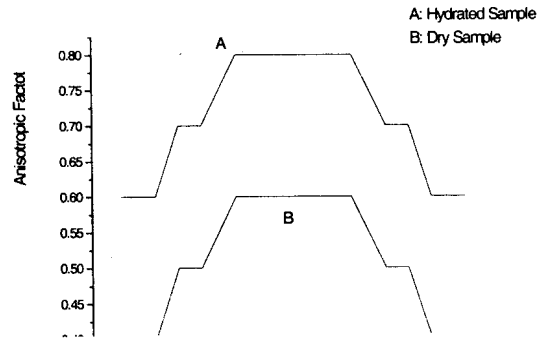


Fig:6. Anisotropic factor "g" as a function of wavelength.

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 suitable information for skin tissue modeling. Our experimental data and the investigations were performed at 400-700nm (visible & 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 autofluresence 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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