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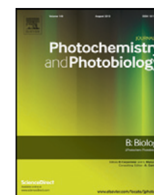

The optical properties of mouse skin in the visible and near infrared spectral regions

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ABSTRACT

Visible and near-infrared radiation is now widely employed in health science and technology. Pre-clinical trials are still essential to allow appropriate translation of optical methods into clinical practice. Our results stress the importance of considering the mouse strain and gender when planning pre-clinical experiments that depend on light–skin interactions. Here, we evaluated the optical properties of depilated albino and pigmented mouse skin using reproducible methods to determine parameters that have wide applicability in biomedical optics. Light penetration depth (δ), absorption (μ_a), reduced scattering (μ'_s) and reduced attenuation (μ'_t) coefficients were calculated using the Kubelka–Munk model of photon transport and spectrophotometric measurements. Within a broad wavelength coverage (400–1400 nm), the main optical tissue interactions of visible and near infrared radiation could be inferred. Histological analysis was performed to correlate the findings with tissue composition and structure. Disperse melanin granules present in depilated pigmented mouse skin were shown to be irrelevant for light absorption. Gender mostly affected optical properties in the visible range due to variations in blood and abundance of dense connective tissue. On the other hand, mouse strains could produce more variations in the hydration level of skin, leading to changes in absorption in the infrared spectral region. A spectral region of minimal light attenuation, commonly referred as the "optical window", was observed between 600 and 1350 nm.

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1. Introduction

Over the last few decades, light has been widely employed in life sciences [1]. Specifically in medicine and dentistry, light has become a powerful alternative approach for non-invasive therapy and diagnosis [2,3]. Hence many pre-clinical studies employ light either for detection of pathology or to induce biological responses. There is a need to clearly understand light–tissue interactions as an important step before translation into clinical practice [4]. Mouse models have been widely employed in pre-clinical studies, even though the structure of mouse skin has some dissimilarities with human skin. Indeed, since many experimental approaches have first been tested in mice, some recent studies have explored the variation in the optical properties of mouse skin with respect to gender [5], strain and age [6], and pathological conditions, such as inflammatory processes [7]. Skin is the most important optical barrier in non-invasive optical techniques and in many cases it is the target tissue. Therefore, skin optics should be well characterized to optimize the use of light-based techniques [8].

Theoretical approaches have not succeeded in achieving a satisfactory modeling approach for such a complex tissue, even though many models have been tested with varying degrees of success [9]. Kubelka–Munk (KM) photon transport theory is a simple, useful and well-accepted analytical model to provide a simple quantitative description of general tissue optics. It is a special case of the "many flux theory" describing two opposite distinguishable fluxes propagating in one dimension: one flux corresponding to the incident beam propagation, and the other to the resultant backscattering [10,11]. The main advantage of this model is its ability to be simply described by two differential equations, one corresponding to each flux, and the solutions are only dependent on the following parameters: sample thickness (D), the diffuse transmittance (T_d) and the reflectance (R_d). The light fluxes are subject to both elastic scattering and single photon absorption events and the probability of

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occurrence of these events is directly proportional to the KM scattering (S_{KM}) and absorption (A_{KM}) coefficients, respectively.

In the present work, we evaluated the optical behavior of freshly excised pieces of skin from male and female mice of two different strains: one albino strain (BALB/c) and another melanin pigmented strain (C57BL/6). Despite the fact that the melanin level is generally supposed to be low in C57BL/6 mice skin [6] it is recognized that skin pigmentation strongly influences the optical properties of tissues [12]. We analyzed a very broad spectral range (from 400 to 1400 nm), using the Kubelka–Munk model of photon transport and spectrophotometric measurements, to precisely describe the optical properties of mouse skin in terms of the optical coefficients μ_a, μ'_s, μ'_t and penetration depth (δ).

2. Materials & Methods

2.1. Animals

We used 4 to 6 weeks old healthy mice, 5 males and 5 females from each strain (C57BL/6 and BALB/c), for *ex vivo* skin sampling. Before the experiments, all animals were individually housed in acrylic plastic isolators in a 12 h light/dark cycle and fed with granulated food and water *ad libitum*. All animal procedures, care, and handling were carried out according to the ethical principles of animal experimentation formulated by the Brazilian College for Animal Experimentation (COBEA) and were approved – protocol 95/11 – by the local Ethics Committee on Animal Research and Care of IPEN/CNEN-SP.

Animals were euthanized by cervical dislocation, and hair excess on the dorsal region was carefully removed using an electric shaver. In order to minimize light interaction with any remaining fur, skin was depilated with a thioglycolate-based chemical (Veet Cream®, Reckitt Benckiser, Brazil). Samples were excluded if any sign of surface damage or inflammation was observable.

Samples were harvested from the dorsal region since this is a large region of skin with a homogenous structure, and is also the most frequently used region in mouse models. Skin from other regions such as the abdomen may have less homogeneous structures due to the presence of anatomical irregularities, appendages and other organs, such as mammary glands, that would negatively affect the precision of our interpretations. Skin pieces containing all tissue layers (*i.e.* from epidermis to subcutaneous tissue) of 2×2 cm were then carefully detached from fascia and excised using surgical scissors. To provide mechanical support, each sample was positioned between two microscopy slides under minimal pressure as shown in Fig. 1. Before the sample was positioned between the microscope glass slides, the epidermal surface was slightly moistened by a wet piece of cotton to minimize the effect of mismatched boundary conditions. Sample full thicknesses were measured by a hand caliper, subtracting the microscope slide thickness. To avoid possible artifacts due to sample compression or histological preparation, we used this thickness information to calculate the optical coefficients.

2.2. Spectroscopy Analysis

Immediately after skin excision, spectroscopic measurements were carried out by a commercial spectrophotometer system (Cary 5000, Agilent, Australia) coupled to a single integrating sphere (Internal DRA-2500, Agilent, Australia). The spectra for transmittance and reflectance were measured at wavelengths ranging from 400 nm to 1400 nm. For experimental reproducibility, we avoided any sample folding and minimized the experimental time to avoid any sample deterioration due to possible dehydration and/or oxidation of tissue chromophores. Each measurement run took around 3 min of acquisition time. No tissue changes were visible after these measurements.

Transmittance measurements were obtained by placing the samples in front of the sample light beam (position “a”, in Fig. 1) and the reflectance spectra were obtained by shifting the sample to position “b”. For total transmittance measurements we positioned samples on port “a”, and port “b” was closed to backscatter collimated transmittance. The sample beam was positioned at the center of the samples for reproducibility. During measurements, the rectangular sample beam dimensions were 1 cm high and <0.5 mm wide and the incident optical intensity <1 mW/cm^2. Any thermal effects were considered negligible.

Since the fresh skin samples were placed between two microscopy slides (Fig. 1), the Fresnel reflection provided from the air/glass/water/glass/air interfaces were subtracted for position “a” and “b” in a baseline calibration. For baseline calibrations, we used two microscope slides with a thin layer of water between the slides.

The optical parameters A_{KM} and S_{KM} were determined using the one-dimensional, two-flux KM model [10,13]. The theoretical model provides rather simple mathematical formulations to extract the optical parameters from the measured values of T_d and R_d.

$$A_{KM} = (x-1)S_{KM} \quad (1)$$

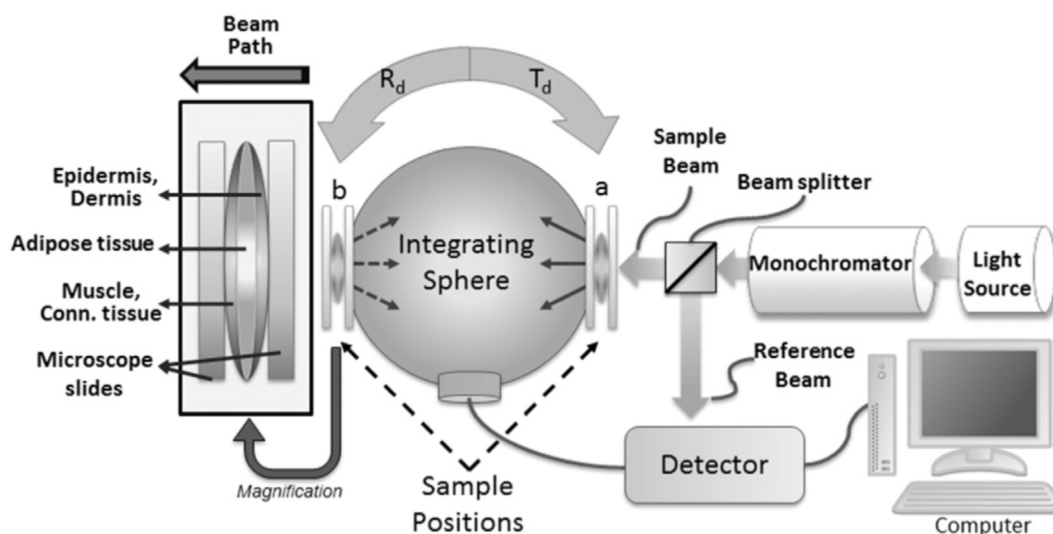

Fig. 1. Schematic diagram of the commercial spectrometer system coupled to a single integrating sphere. To provide mechanical support, each sample was placed in between two glass microscopy slides. The transmittance spectra were obtained by placing the sample at position “a” with port “b” closed, and reflectance spectra were acquired shifting the sample to position “b”. For all measurements, the sample beam was directed in the epidermis–dermis direction.

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