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Monte Carlo simulation of near infrared autofluorescence measurements of *in vivo* skin

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ABSTRACT

The autofluorescence properties of normal human skin in the near-infrared (NIR) spectral range were studied using Monte Carlo simulation. The light-tissue interactions including scattering, absorption and anisotropy propagation of the regenerated autofluorescence photons in the skin tissue were taken into account in the theoretical modeling. Skin was represented as a turbid seven-layered medium. To facilitate the simulation, *ex vivo* NIR autofluorescence spectra and images from different skin layers were measured from frozen skin vertical sections to define the intrinsic fluorescence properties. Monte Carlo simulation was then used to study how the intrinsic fluorescence spectra were distorted by the tissue reabsorption and scattering during *in vivo* measurements. We found that the reconstructed model skin spectra were in good agreement with the measured *in vivo* skin spectra from the same anatomical site as the *ex vivo* tissue sections, demonstrating the usefulness of this modeling. We also found that difference exists over the melanin fluorescent wavelength range (880–910 nm) between the simulated spectrum and the measured *in vivo* skin spectrum from a different anatomical site. This difference suggests that melanin contents may affect *in vivo* skin autofluorescence properties, which deserves further investigation.

1. Introduction

Optical techniques have recently received considerable attention in the fields of biomedical diagnostics and monitoring [1,2]. The autofluorescence imaging and spectroscopy for *in vivo* and *ex vivo* characterization of biological materials has been well established. These methods are often based on intrinsic fluorophores, such as porphyrin, tryptophan, tyrosin, NADH, and flavins [3,4]. Autofluorescence emissions are affected by the excitation light distribution inside the tissue. The observed fluorescence intensity of a biological tissue is also a function of its fluorophore concentration, extinction coefficient (absorbing power) at the excitation wavelength, and quantum yield at the emission wavelength. The tissue re-absorption and scattering to the emitted fluorescence photons during the escape process are also important factors. A complete understanding of the *in vivo* spectrum must therefore take into account all the above factors.

Modeling of fluorescence measurements in the UV/visible wavelength range has been reported previously [5–10] including our own work [11–13]. In this paper, we built an optical model to study the normal skin fluorescence in the near infrared (NIR) wavelength range in response to the increased interests of using NIR autofluorescence for tissue analysis and diagnosis [14,15]. Due to decreased tissue absorption and its increased penetration depth, NIR autofluorescence is preferred for certain applications, e.g. the detection of melanin distributions in human skin *in vivo* [16–18]. In this modeling work, transport parameters of skin tissue in the NIR wavelength range were compiled from a number of publications [19–22]. We modeled the *in vivo* autofluorescence measurements of normal human skin in the NIR range by Monte Carlo simulation. During the modeling, the photon absorption was recorded to calculate the extinction coefficient at 785 nm excitation wavelength using the Monte Carlo code from Wang and Jacques directly [23,24]. The code was also modified to simulate the fluorescence escape process. Fluorescence spectra and images of *ex vivo* normal skin sections were measured to quantify the fluorophores density and intrinsic spectra of different layers. The NIR fluorescence images obtained demonstrated that the skin fluorophore distribution was not uniform, but had a layered structure similar to the visible fluorescence distribution in our previous

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work [25,26]. This indicates that all previous theoretical modeling framework in the visible wavelength range developed in our lab [11–13] are still appropriate for the NIR wavelength range modeling. However, because the types of fluorophores are different among NIR, Visible and UV light excitation, the model has to be revised for the NIR wavelength range. We also measured the NIR fluorescence of human normal skin *in vivo*, and found that the simulated fluorescence spectra was in good agreement with the measured *in vivo* skin spectra. We believe that this modeling work will help better understanding the NIR skin fluorescence properties.

2. Materials and methods

2.1. In vivo measurement of human skin NIR fluorescence

The setup used for *in vivo* fluorescence measurement has been described in detail elsewhere [16]. It consists of a 785 nm diode laser (BRW-785-1.0-100-0.22-SMA, B&W, Tek Inc.), a transmissive imaging spectrograph (HoloSpec-f/2.2-NIR, Kaiser), a NIR-optimized, back illuminated, deep-depletion, CCD detector (LN/CCD-1024EhRB, Princeton Instruments), and a specially designed skin probe. The skin probe consists of a signal collection arm and a separate illumination arm. It is especially designed to maximize the collection efficiency of inherently weak signals while reducing the interference of Rayleigh-scattered light and fiber fluorescence. The NIR autofluorescence signal, which is collected by the skin probe, is delivered to the transmissive spectrograph through a customized optical fiber bundle. In order to improve light collection efficiency and maintains high spectral resolution, the fiber bundle consists of 58 100 μm core-diameter fibers [16].The diffusely reflected laser light was rejected by a 785 nm notch filter. Each NIR spectra can be acquired over the range 820–920 nm, displayed on the computer screen in real time, and saved for further analysis. In this study, we measured the NIR autofluorescence spectra of earlobe from six volunteers.

2.2. Ex vivo measurements of NIR fluorescence from excised human skin tissue sections

A home-made modular microspectroscopy system is used to measure *ex vivo* skin tissue sections [27]. The system setup is schematically shown in Fig. 1. To be briefly, its major components include a fiber-coupled diode laser (BRW-785-1.0-100-0.22-SMA, B&W, Tek Inc.), an inverted microscope (Diaphot, Nikon), and a transmissive imaging spectrograph (HoloSpec-f/2.2-NIR, Kaiser) and a CCD camera (Spec-10:100BR/LN, Princeton Instruments). A fiber bundle was designed to deliver the signals to the spectrograph. The fiber bundle was composed of 37 40 μm core-diameter optical fibers, which was packed into a circular area of 100-μm in diameter in the microscope side, and aligned into two closely-packed columns in the spectrograph side to enhance the detection of the inherently low autofluorescence (and Raman) signals. In order to battle the background fluorescence from the system, conventional normal illumination was replaced by oblique illumination at 45° and the microscope slides were coated with gold. These modifications substantially reduced the background from the slides and the system so that autofluorescence (and Raman) spectra from skin tissue sections can be obtained. The autofluorescence and Raman signal could be collected over the range of 837–914 nm. The Raman signal overlapped on top of the fluorescence signal is narrow and weak peaks that can be removed automatically by a special polynomial fitting method [28] – the Vancouver Raman Algorithm. Fresh skin samples were frozen and cut into sections of about 20 μm thick (unstained and unfixed) and then placed on gold coated microscope slides for the examination. NIR fluorescence images were also recorded using a computer-controlled NIR-sensitive CCD camera (Alta® U1, Apogee Instruments), which was connected to the microscope.

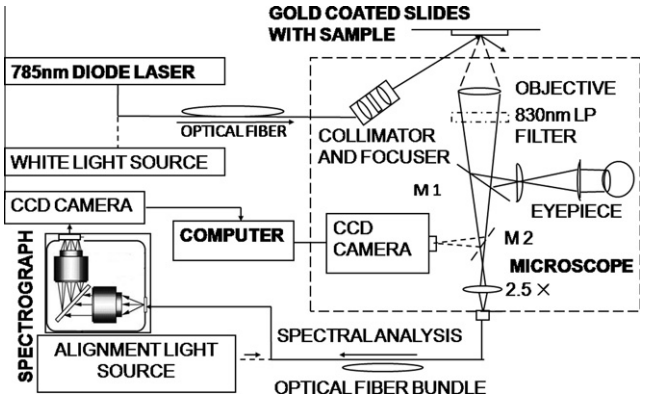

Fig. 1. Setup of the home-made microspectroscopy system.

2.3. Optical model of normal human skin

We have published a systematic study on the modeling of light propagation and visible fluorescence transport in normal human skin [11,12,26]. The skin is a layered structure interspersed with various appendages such as hair follicles, sweat glands, and sebaceous glands, which we will ignore for simplicity but without loss of generality. A seven-layer skin optical model for normal skin and a method of determining the optical parameters of different skin layers are used in the model [11,12,26]. In this study, we designed a seven-layer skin optical model in the NIR wavelength range with transport parameters compiled from Salomatina et al. [22] and Meglinski and Matcher [29]. Table 1 outlines this seven-layer model including thickness (d), refractive index (n), and the optical transport parameters (absorption coefficient, μ_a, scattering coefficient, μ_s, scattering anisotropy, g) at 785 nm for each skin layer. For this study, 785 nm is the wavelength of the laser light used for fluorescence excitation. The refractive index of the ambient medium above the 7-layer tissue is 1.0, which is assumed to be air, and the ambient medium below the 7-layer tissue is 1.46, which is assumed to be subcutaneous fat.

The optical properties of normal skin have been investigated by several groups [29–31]. However, the data for the optical properties of epidermis and stratum corneum in the NIR wavelength range are rare [32,33], since most of the investigators studied either the optical properties of dermis and subcutaneous fat [33], or reported on the combined optical properties of epidermis and dermis [21,34]. In our model, we assumed that the anisotropy factor g was 0.8 and the refractive index to be 1.4 for all the skin layers in the entire spectral range investigated [22]. It is well established that skin absorption in the NIR spectral region is dominated by oxy-/deoxyhaemoglobin and water [35–37]. Since the

Table 1
The seven-layer skin optical model for normal skin. The transport parameters (μ_a, μ_s, g, n) for 785 nm excitation wavelength.

Layer	d (μm)	n	μ_s (cm^{-1})	μ_a (cm^{-1})	g
Air	–	1.0	–	–	–
Stratum corneum	10	1.4	189.5	0.0089	0.8
Epidermis	80	1.4	189.5	1.9	0.8
Papillary dermis	100	1.4	116.5	1.3	0.8
Upper blood plexus	80	1.39	154.85	1.5875	0.818
Reticular dermis	1500	1.4	116.5	1.3	0.8
Deep blood plexus	70	1.34	461.65	3.8875	0.962
Dermis	160	1.4	116.5	1.3	0.8
Subcutaneous fat	–	1.46	–	–	–