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Correction for tissue optical properties enables quantitative skin fluorescence measurements using multi-diameter single fiber reflectance spectroscopy

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ABSTRACT

Background and objective: Fluorescence measurements in the skin are very much affected by absorption and scattering but existing methods to correct for this are not applicable to superficial skin measurements.
Study design/materials and methods: The first use of multiple-diameter single fiber reflectance (MDSFR) and single fiber fluorescence (SFF) spectroscopy in human skin was investigated. MDSFR spectroscopy allows a quantification of the full optical properties in superficial skin (μ_a, μ_s' and γ), which can next be used to retrieve the corrected – intrinsic – fluorescence of a fluorophore $Q\mu_{ax}^{f}$. Our goal was to investigate the importance of such correction for individual patients. We studied this in 22 patients undergoing photodynamic therapy (PDT) for actinic keratosis.
Results: The magnitude of correction of fluorescence was around 4 (for both autofluorescence and protoporphyrin IX). Moreover, it was variable between patients, but also within patients over the course of fractionated aminolevulinic acid PDT (range 2.7–7.5). Patients also varied in the amount of protoporphyrin IX synthesis, photobleaching percentages and resynthesis (>100× difference between the lowest and highest PpIX synthesis). The autofluorescence was lower in actinic keratosis than contralateral normal skin (0.0032 versus 0.0052; $P < 0.0005$).
Conclusions: Our results clearly demonstrate the importance of correcting the measured fluorescence for optical properties, because these vary considerably between individual patients and also during PDT. Protoporphyrin IX synthesis and photobleaching kinetics allow monitoring clinical PDT which facilitates individual-based PDT dosing and improvement of clinical treatment protocols. Furthermore, the skin autofluorescence can be relevant for diagnostic use in the skin, but it may also be interesting because of its association with several internal diseases.

1. Introduction

Fluorescence spectroscopy can be used to measure the concentration of common fluorophores in the skin such as NAD(H), collagen, elastin, keratin, and advanced glycation end-products [1–4]. The combined emission of these fluorophores constitutes the skin autofluorescence, and higher levels are correlated with cardiovascular events, diabetes mellitus, chronic pulmonary disease, renal disease, liver cirrhosis and inflammatory diseases [5–11]. Fluorescence spectroscopy can also be used to measure protoporphyrin IX (PpIX) concentrations during photodynamic therapy (PDT). Such measurements are important because it is well documented that PpIX fluorescence kinetics can help predict PDT outcome [12–17]. However, the problem with existing fluorescence measurements is that the detected fluorescence signals are critically dependent on the unknown optical properties of the tissue.

Abbreviations: MDSFR, multiple diameter single fiber reflectance spectroscopy; SFF, single fiber fluorescence; ALA, 5-aminolevulinic acid; PDT, photodynamic therapy; PpIX, protoporphrin IX.

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In fluorescence spectroscopy both the excitation and emission light undergoes absorption and scattering and, as a result, the tissue optical properties influence the measured fluorescence intensity. Therefore, a crucial step to quantitate fluorescence is to correct it for these optical properties. Various investigators have tried to achieve this, but have not yet recovered the intrinsic fluorescence [18–24]. A review on tissue fluorescence measurement methods concludes that there is a clear need for the development of new correction techniques [25]. In the current paper a recently developed correction technique is described that enables quantitative fluorescence measurements in a superficial layer of tissue.

The superficial nature of the volume over which the optical properties are measured is a crucial consideration for quantitative optical spectroscopy in the skin. The epidermis and superficial part of the dermis are the areas in which most of the fluorophores are present in case of PDT, but also where different patients, lesions and skin diseases differ most in optical properties. Measuring these optical properties in such a superficial sampling volume poses a significant challenge.

One solution is to make use of an optical technique in which the path-length of light can be controlled, such as single fiber reflectance (SFR) spectroscopy [26–30]. A SFR spectrum contains the combined information on how much light has been absorbed and scattered. From such a reflectance spectrum the tissue absorption coefficient, μ_a (mm^{-1}) can be quantified without prior knowledge of the scattering properties [29]. Decomposition of μ_a into constituent spectra of known tissue chromophores yields accurate measurements of various physiological parameters. The result of measuring so superficially is that the number of scattering events is relatively small. This means that the angle in which the light is scattered becomes critical. This scattering angle follows a probability distribution that is described by the tissue phase function, which must be characterized in order to measure the reduced scattering coefficient μ_s' (mm^{-1}). We have recently demonstrated that successive SFR measurements using two or more carefully chosen fiber diameters enables to quantification of both the phase function parameter $\gamma = (1 - g_2/1 - g_1)$ and μ_s' [31–33]. We have termed this technique multiple diameter single fiber reflectance (MDSFR) spectroscopy. The mathematical model to extract the optical properties from a spectrum has been validated using tissue-mimicking phantoms and computer simulations [32,33].

The optical properties that are measured using MDSFR can be used to correct a subsequent fluorescence measurement [34,35]. This corrected – intrinsic – fluorescence is given as the product of the absorption coefficient of the fluorophore for the excitation wavelength $\mu_{a,x}^f$ and the quantum efficiency of the fluorophore across the emission spectrum Q (–). We term this method single fiber fluorescence (SFF) spectroscopy and have recently applied it for measuring intrinsic fluorescence in pre-clinical models [36].

This study describes for the first time the use of MDSFR and SFF spectroscopy in human skin. Our aim is to introduce the technique in a clinical setting, demonstrate its ability to quantify fluorescence and show its potential value in dermatology and general medicine. We chose to perform MDSFR/SFF in patients undergoing fractionated aminolevulinic acid (ALA) PDT for keratinocytic intraepithelial neoplasia (KIN; either actinic keratosis or Bowen's disease). This is useful because fluorescence measurements in PDT have previously been proven to be important in predicting patient-specific outcomes, illustrating the potential clinical importance of quantitative fluorescence. Both skin autofluorescence and PpIX fluorescence were measured. Since optical properties are known to change during PDT [37–40], we measured PpIX fluorescence at various time-points during PDT.

2. Materials and methods

2.1. MDSFR and SFF setup

To obtain a reflectance spectrum, light from a tungsten halogen lamp was directed on the skin through a probe containing 2 single fibers (0.4 and 0.8 mm diameter). This choice is related to the fact that the sampling volume is on the order of the fiber diameter [30,41] and ensures that both the epidermis and superficial dermis are sampled [38]. After these sequential MDSFR measurements, a SSF fluorescence spectrum was taken using the 0.8 mm fiber under 405 nm excitation. The reflectance and fluorescence spectra were detected by three spectrometers, one for each fiber to measure reflectance and one to measure fluorescence with a filter blocking the laser light (Fig. 1). Spectrometers and light sources were controlled by a custom made LabView program (NI, LabView v7.1) as described previously [42]. A combined MDSFR/SFF measurement took approximately 3 s.

Fig. 2 shows an example of typical MDSFR spectra. In this figure the percentage of incident light collected by each optical fiber is plotted as a function of wavelength and includes the MDSFR model fit to the data. For both fibers the overall shape is characterized by a signal that decreases with wavelength (gray line) that is attributable to the background scattering, which was modeled by a power law function [42]. Note that the reflectance is higher for the 0.8 mm fiber diameter, because the collection area is larger.

2.2. Reflectance spectral analysis

To retrieve quantitative data on the tissue optical properties and the intrinsic fluorescence, spectral analysis was performed using the same fitting model for the data as described previously. For a detailed description of this procedure we refer to these articles [31–33,36,43]. A simplified stepwise summary is described below.

First the tissue absorption properties are calculated, defined by the absorption coefficient μ_a (mm^{-1}). For each wavelength this is the sum of the known absorption coefficients of (de)oxygenized hemoglobin, melanin, bilirubin and beta-carotene. This results in the following measured parameters of the

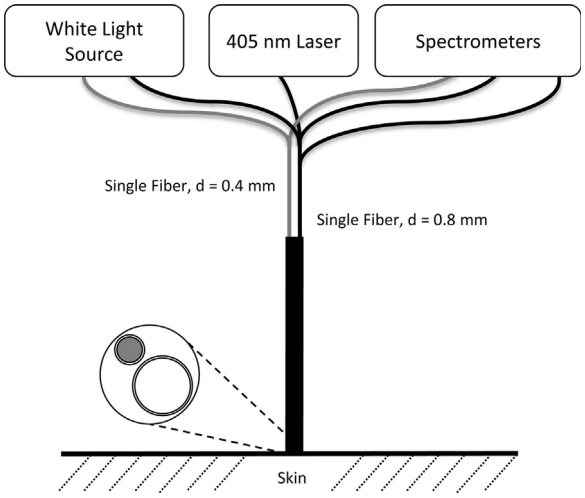

Fig. 1. Schematic representation of the measurement setup. White light reflectance and fluorescence (405 nm laser excitation light) spectra were acquired using a single probe that contained two fibers of 0.4 and 0.8 mm. The 0.8 mm fiber was used for both fluorescence and reflectance spectroscopy while the 0.4 mm fiber performed reflectance spectroscopy only.