Quantitative optical spectroscopy in the skin

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The human eye is the primary optical diagnostic instrument used in dermatological practice. It is the golden standard for the visual inspection of skin colour, erythema, scaliness, shininess, oiliness, edema, and the presence of specific chromophores, such as blood in the superficial vasculature or the appearance of a border in pigmented lesions. Distinguishing between these attributes in healthy and diseased skin forms the basis of clinical diagnosis in dermatology. A visual assessment can be augmented by the use of simple magnifying devices, such as the dermascope, which may aid the diagnosis of skin lesions. The human eye is also a critical factor in the determination of a histological diagnosis where visible differences in (sub-) cellular morphology are the central component of the microscopic analysis of skin biopsies.

Despite the ease of a visual skin inspection, it is important to recognize that in many cases the eye or a simple imaging device is not well suited for distinguishing subtle differences in, for example, hue and/or scaliness. The subjectivity of visual analysis remains the primary challenge with the majority of clinical diagnoses. In order to better understand the nature of visual skin assessments and to identify the potential for more advanced approaches, it is important to consider (a) the underlying interaction between light and the physio-anatomical structure of the skin and (b) the way in which light might be used to quantify the constituents of tissues such as skin.

Cells in the skin are not visible to the naked eye but contribute to its visual appearance as a result of their interactions with incident light. From an optical viewpoint, the dominant effects produced at this microscopic scale are light scattering and absorption. At the macroscopic scale, these are quantified by the absorption and scattering coefficients of tissue. Their magnitude depends on the size and shape of the (sub-) cellular components in the tissue, as well as their optical parameters such as differences in their refractive index. For example, organelles and fibers of the cytoskeleton found within cells behave as strong light scattering centers. These scattering centers change the direction of the light as it travels through the tissue, which alters the spatial distribution of the light without light being absorbed. Certain specific cell types (such as melanocytes and erythrocytes)
contain chromophores (melanin and hemoglobin) that are responsible for the absorption properties of the skin. A separate class of chromophores (such as collagen and elastin) absorb light of a given wavelength and emit longer wavelength radiation as fluorescence. These molecules are called fluorophores. Two important aspects of these processes (scattering, absorption and fluorescence) are that their magnitudes are strongly dependent on the wavelength of light and the contributions to these processes by various tissue components overlap significantly in the visible spectrum. As a result, accurate identification and separation of tissue components requires a spectroscopic approach.

There is a further complication; even if the concentrations of chromophores, fluorophores, and scattering centers are known for a specific tissue, quantification of the tissue optical properties cannot be achieved unless one knows how far the light has traveled in the tissue prior to detection. During visual skin inspection, light entering the eye (or a simple imaging device placed at a distance) from an illuminated skin surface has travelled through an unknown distribution of paths within the skin. These ‘path-lengths’ are dependent on the optical properties of the skin, i.e. on the absorption and scattering coefficients of the skin. These are in turn related to the microscopic concentration of absorbers and scattering centers and, as described above, are strongly dependent on the wavelength of light. Accurate knowledge of the path-length of light in the tissue is critical because it is this knowledge that allows calculation of the magnitude of the absorption and scattering coefficients from changes in the light returning from the skin. As a result of these variable, unknown, wavelength-dependent path-lengths, it is impossible to quantify the constituents of tissue using visual inspection or a simple imaging device.

To overcome this problem, research on quantitative measurements has focused on the use of optical techniques in which the path-length of light can be controlled. For the majority of cases, this involves placing an optical probe on the surface of the skin through which light enters the tissue and is collected at a certain distance away. The use of a known distance between light source and light detection is important for two reasons. First, it determines the volume over which optical signals are averaged, which is an important consideration for the interrogation of skin lesions and their surroundings. Second, by using a known separation distance between the light source and the light detection, theoretical models can be built to describe the complex interaction of light with tissue over this distance. By applying these theoretical models to the light measured by the optical probe, the tissue optical properties can be quantified.

Given the fundamental difficulties of performing quantitative measurements of chromophore, fluorophore or scatterer concentrations in tissue, it is no wonder that the human eye faces significant challenges. While erythema is (relatively) easy to identify, a visual inspection cannot determine the relative contributions from oxy- and de-oxymegoglobin or quantify the average diameter of blood vessels in the superficial vasculature. Nor can the eye quantify the presence of low concentrations of absorbers such as bilirubin and beta-carotene, particularly in different skin types that contain variable quantities of melanin. A Wood's lamp can be used as a method for assessing the presence and distribution of melanin in pigmented lesions and for exciting endogenous and exogenous fluorophores in the skin. However, without any knowledge of the tissue absorption and scattering coefficients, these types of measurements are at best qualitative, and at worst can result in very misleading signals, particularly in the presence of skin that contains a significant amount of blood and/or has unknown scattering properties.

Despite the complexity of the challenges described above, it is important to stress that the successful implementation of quantitative optical spectroscopy in the skin may have significant clinical benefits: Abnormalities measured in the skin may reflect systemic diseases of internal organs and serve as a non-invasive diagnostic tool or aid in the diagnosis of skin diseases. Physiological parameters such as blood volume and micro-vascular blood saturation may be related to the presence of cancer or other abnormalities of the cutaneous vasculature. Quantitative information on the presence of other chromophores such as bilirubin and beta-carotene may provide information on underlying metabolic diseases. Using optical spectroscopy to measure the optical properties of the skin, these properties can be used to perform quantitative fluorescence spectroscopy in which the intrinsic fluorescence is recovered from the skin. Such quantitative fluorescence measurements may be clinically important. For example, the measurement of certain endogenous fluorophores, such as NADH, has been suggested as a method of monitoring the redox state of cells and tissues. The measurement of collagen and elastin may have clinical relevance in quantifying the structural properties of the skin. Other investigators have reported that skin autofluorescence can be used to measure advanced glycation end products (AGEs), such as glucuronic acid, 3-indoxyl sulfate, 3-hydroxybutyrate, phenol sulfate, and pentosidine, which have been linked to both diabetic complications and cardiovascular disease. Similarly the quantification of exogenous fluorophores such as aminolevulinic acid (ALA) -induced protoporphyrin IX (PpIX) can be used for diagnosing skin lesions or to monitor PDT and predict PDT outcome.
QUANTITATIVE REFLECTANCE AND FLUORESCENCE SPECTROSCOPY

Over the past 10 years, the Center for Optical Diagnostics and Therapy of the Erasmus MC has been developing methods of performing quantitative fiber optic spectroscopy. These methods use approaches where the distance between the point where light enters the tissue and light is collected is small. One recent example of this approach, involves the use of a single fiber for the delivery and collection of light, is called single fiber reflectance spectroscopy (SFR). We have developed mathematical models to describe the underlying interaction of light with tissue. The sampling volume can be tailored to a specific clinical application by varying the diameter of the optical fiber(s) that are used [range in sampling depth 0.1 – 1 mm]. Using knowledge of the sampling volume, we have developed algorithms that can be used to perform quantitative reflectance and fluorescence spectroscopy. A significant advantage of SFR is that it can be implemented utilizing two or more fiber diameters in a single probe, which enables a complete determination of tissue optical properties. This technique, termed multi-diameter single fiber reflectance spectroscopy (MDSFR), can then be used to recover a fully quantitative measure of intrinsic fluorescence. Another major advantage of this approach is a consequence of having overlapping delivery and collection light fields. In this situation, the distribution of angles at which the light scatters becomes an essential factor in the mathematical models that we have developed to describe light scattering. This is critical because there is evidence that measuring these types of scattering properties may be diagnostically valuable: changes in the angular distribution of scattered light can be directly correlated to changes in the nanoscale cellular organization or ultrastructure of tissue.

Alterations in ultrastructure, invisible in conventional histology, are known to occur during early cancer development and are hypothesized to result from cytoskeletal dysregulation. This dysregulation alters the local concentration, shape and size distribution of subcellular structures such as actin filaments, ribosomes, mitochondria, chromatin, DNA and RNA, are implicated in early carcinogenesis. We therefore expect that MDSFR spectroscopy will be useful in quantifying disease-related ultrastructural alterations in pre-cancerous lesions that may be related to disease progression.

CLINICAL MEASUREMENTS

Over the past decade our group has performed reflectance spectroscopy in a wide range of predominantly internal organs including the oral cavity, respiratory tract, gastrointestinal tract and in the genitourinary system. More recently we have acquired quantitative reflectance and fluorescence spectra from the skin in a number of settings. Here we have selected 2 clinical measurement procedures to illustrate the potential of quantitative optical spectroscopy in the skin; (i) MDSFR in actinic keratosis (AK), (ii) quantitative single fiber fluorescence spectroscopy (SFFL) in patients with AK undergoing aminolevulinic acid photodynamic therapy (ALA-PDT).

MDSFR in actinic keratosis
An MDSFR system consisting of a single fiber probe containing 2 single fibers with diameters of 0.4 and 0.8 mm and a halogen light source for illumination is coupled with two spectrographs for detection. The coordination of illumination, detection and appropriate calibration steps is performed by a laptop PC. Figure 2 shows an example of MDSFR spectra acquired from AK. The percentage of incident light collected by each optical fiber is plotted as a function of wavelength with an associated mathematical model fit to the data. In each case the overall shape of the SFR signal is characterized by a signal that decreases with wavelength (grey line) that is attributable to the wavelength-dependent scattering of light in tissue.

The presence of chromophores is observed as a reduction in the amount of scattered light that is collected and characterized by dips in the reflectance signal illustrated in figure 2. In this example the absorption due to melanin and particularly hemoglobin, which has characteristic features around 400 nm and between 500 and 600 nm, are evident in the SFR spectra. The fact that we have developed and validated a theoretical
model that accurately describes how the presence of absorption and scattering influences the SFR signals collected from different fiber diameters means it is possible to extract the concentrations of chromophores and parameters that quantify light scattering in skin. The blood volume fraction (bvf) in AK is proportional to the depth of the signal dips observed below 600 nm corresponding to the absorption of light by hemoglobin. The shape of the absorption dips in the 500-600 nm wavelength band is characteristic of the StO2, with a single-dip associated with deoxygenated blood and a double-dip associated with oxygenated blood. The relative depth of the signal dips around 420 nm vs. 500-600 nm is clearly visible over the autofluorescence background. Again this intrinsic fluorescence of PpIX, after correction for the influence of tissue optical properties and background autofluorescence, is directly related to the concentration of PpIX in cells with a functional heme biosynthesis pathway.

Figure 3 shows the wavelength dependence of \( \gamma \), which is a measure of the direction of scattering in tissue and the reduced scattering coefficient \( \mu'_s \).

**Figure 3. The wavelength dependence of \( \gamma \), which is a measure of the direction of scattering in tissue and the reduced scattering coefficient \( \mu'_s \).**

Future studies
An MDSFR system that utilizes 3 concentric optical fibers bundles is currently being used to acquire data from patients in the Erasmus Rotterdam Health Research (ERGO) population study. This combined MDSFR/SFFL device will be used to quantitatively monitor the changes in intrinsic fluorescence in individual lesions. In the future this may lead to predictors of efficacy that have clinical relevance.
skin concentrations of melanin, beta-carotene, blood volume fraction, blood oxygen saturation, microvessel diameter, and scattering center concentration and shape) leads to clinical indicators of local and systemic disease.

REFERENCES


De complete literatuurlijst is, vanaf drie weken na publicatie in dit tijdschrift, te vinden op www.huidarts.nl
SAMENVATTING
Het oog van de dermatoloog is zijn of haar belangrijkste instrument voor het stellen van een diagnose. Hoewel het praktisch is dat men een dergelijk instrument direct ter beschikking heeft, is het belangrijk te beseffen dat onze ogen in feite maar beperkte optische instrumenten zijn. Subtiele of microscopische verschillen tussen huidziekten kunnen onze ogen maar slecht onderscheiden. In dit artikel beschrijven we de interactie tussen licht en de fysioanatomische structuur van de huid, hoe dit onze visuele waarneming beïnvloedt (en beperkt), en hoe geavanceerdere optische technieken kunnen bijdragen aan een beter onderscheidend vermogen. Hierbij wordt de nadruk gelegd op de mogelijkheden van een recent ontwikkelde optische techniek, namelijk reflectie- en fluorescencie-spectroscopie door middel van twee of meer optische fibers met verschillende diameters. Gecombineerd met theoretische modellen die de interactie tussen licht en weefsel beschrijven, is het nu mogelijk om de absorptie-, verstrooings- en fluorescencie eigenschappen van de huid te kwantificeren. Het potentieel van deze techniek wordt geïllustreerd aan de hand van patiënten met actinische keratose die optische diagnostiek en ALA-PDT ondergaan. Tevens wordt benadrukt hoe ze geïmplementeerd kunnen worden in longitudinaal epidemiologisch onderzoek.

SUMMARY
The human eye is the primary optical diagnostic instrument used in dermatological practice. Despite the ease of a visual skin inspection, it is important to recognise that in many cases the eye or a simple imaging device is not well suited for distinguishing subtle differences in erythema, scaliness or melanin content between dermatoses. The subjectivity of visual analysis remains the primary challenge with the majority of clinical diagnoses. In the present article we describe the underlying interaction between light and the physio-anatomical structure of the skin, the nature and limitations of a visual skin assessment and identify the potential for more advanced approaches. Specifically we make the case for the use of fiber optic spectroscopy, utilising two or more optical fibers with different diameters. When combined with the use of theoretical models, which describe the way that light interacts with tissue, spectroscopy enables the quantification of the absorption, fluorescence and scattering properties of skin. We illustrate the potential benefit of quantitative reflectance and fluorescence spectroscopy in patients with actinic keratosis undergoing optical diagnosis and aminolevulinic acid photodynamic therapy and highlight how these approaches may be implemented in longitudinal population based studies.

Enquête
Cellulitis en erysipelas van de onderste extremiteiten

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Namens de Werkgroep cellulitis en erysipelas van de onderste extremiteiten

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Op de Dermatologendagen 2013 werd de nieuwe conceptrichtlijn Cellulitis en erysipelas van de onderste extremiteiten besproken. Het betreft een multi-disciplinaire richtlijn, die geïnitieerd werd vanuit de NVDV. Daarnaast waren het Nederlands Huisartsen Genootschap, de Nederlandse Internisten Vereniging, de Nederlandse Vereniging voor Heelkunde en de Nederlandse Vereniging voor Microbiologie betrokken bij de ontwikkeling van de richtlijn. Tijdens het uitwerken van de negen uitgangsvragen van de richtlijn bleek voor sommige uitgangsvragen onvoldoende evidence aanwezig om een wetenschappelijk onderbouwd antwoord op de uitgangsvragen te formuleren. Sommige aanbevelingen in de richtlijn zijn dus volledig gebaseerd op de mening van de werkgroep. Daarnaast was er binnen de werkgroep verdeeldheid tussen de verschillende verenigingen over de antimicrobiële behandeling en de plaats van ambulante compressietherapie bij de behandeling van cellulitis en erysipelas in de acute fase en bij recidiverende