In vivo documentation of cutaneous inflammation using spectral imaging

Georgios N. Stamatas
Nikiforos Kollias
Johnson & Johnson Consumer Products Company
Division of Johnson & Johnson Consumer Companies, Inc.
Methods and Models Development
Skillman, New Jersey 08558

Abstract. Typical manifestations of cutaneous inflammation include erythema and edema. While erythema is the result of capillary dilation and local increase of oxygenated hemoglobin concentration, edema is characterized by an increase in extracellular fluid in the dermis, leading to local tissue swelling. Both of these inflammatory reactions are typically graded visually. We demonstrate the potential of spectral imaging as an objective noninvasive method for quantitative documentation of both erythema and edema. As examples of dermatological conditions that exhibit skin inflammation we applied this method on patients suffering from (1) allergic dermatitis (poison ivy rashes), (2) inflammatory acne, and (3) viral infection (herpes zoster). Spectral images are acquired in the visible and near-IR part of the spectrum. Based on a spectral decomposition algorithm, apparent concentrations maps are constructed for oxygenated hemoglobin and deoxyhemoglobin, melanin, optical scattering, and water. In each dermatological condition examined, the concentration maps of oxyhemoglobin and water represent quantitative visualizations of the intensity and extent of erythema and cutaneous edema, correspondingly. We demonstrate that spectral imaging can be used to quantitatively document parameters relevant to skin inflammation. Applications may include monitoring of disease progression as well as screening for efficacy of treatments. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2798704]

Keywords: spectral imaging; erythema; poison ivy; dermatitis; herpes zoster; acne.

Paper 07141SSR received Apr. 16, 2007; revised manuscript received Jul. 4, 2007; accepted for publication Jul. 17, 2007; published online Oct. 19, 2007.

1 Introduction

An inflammatory skin reaction is accompanied by redness (erythema, rubor) of the involved skin area and often with localized tissue swelling (edema, tumor).

In the early phase of acute tissue inflammation, mediators such as histamine, bradykinin, and various prostaglandins relax the smooth muscle layer of arteries and arterioles. The resulting vasodilation leads to increased blood volume in the inflamed area that is clinically perceived as visible erythema. Both oxygenated hemoglobin (oxy-Hb) and deoxygenated hemoglobin (deoxygenated-Hb) in blood absorb strongly in the blue and green parts of the visible spectrum but very weakly in the red part. Therefore light that is remitted from an inflamed skin site appears to be red compared to the surrounding uninvolved skin.

Inflammatory mediators such as histamine, bradykinin, and leukotrienes may also act to increase the permeability of the vascular walls to blood plasma, which could result in increased concentration of extracellular fluid in the tissue. Under physiological conditions, lymphatic vessels drain the excess fluid, but their draining capacity can be exceeded during an inflammatory reaction, which leads to local accumulation of extracellular fluid (exudate).

Cutaneous edema and erythema are typically evaluated clinically using a visual analogue scale. Being subjective, this method depends on the experience of the clinician. Accurate objective documentation through imaging is often required to monitor the evolution of the cutaneous inflammation.

Taking advantage of the particular spectroscopic properties in the visible part of the spectrum of oxy-Hb and deoxy-Hb, their apparent concentrations in the skin can be evaluated by spectroscopic analysis of the light remitted from the skin, a method known as diffuse reflectance spectroscopy (DRS). It has been shown that erythema directly relates to increased apparent concentrations of oxy-Hb, while accumulation of deoxy-Hb relates to blood stasis. Recent advances in digital imaging hardware (optics, detectors, frame-grabbers, etc.) have enabled the development of spectral imaging, which in addition to the spectral information similar to that obtained by DRS, provides 2-D spatial information. More precisely, it provides the means to localize and quantify cutaneous erythema.

In the case of edema the excess fluid is composed primarily of water. The light absorption properties of water in the near IR (NIR) can be used to estimate the severity of the

Address all correspondence to Georgios Stamatas, Johnson & Johnson, 199 Grandview Rd, Skillman, NJ 08558; Tel: (908)874-2542; Fax: (908)874-7205; E-mail: gstamat@cpus.jnj.com
edema reaction.\textsuperscript{16,17} Similar to the case of erythema, spectral imaging provides the means to localize and quantify the cutaneous edema reaction.\textsuperscript{15}

In this paper, we present the potential of spectral imaging as a noninvasive method for quantitative documentation of skin inflammation. We present several examples of diverse dermatological conditions including inflammatory acne, herpes zoster, and rhus dermatitis (poison ivy rashes).

2 Materials and Methods

2.1 Clinical Protocols

All clinical investigations were conducted according to the declaration of Helsinki principles. In the first study, five healthy individuals (male and female, 18 to 60 years of age, fair complexioned, skin types II to IV) without an active skin disease including eczema, urticaria, and history of atopic dermatitis and with positive history to rhus dermatitis volunteered to participate after giving informed consent. Rhus dermatitis was induced with an oleoresin extract.\textsuperscript{18} The oleoresin was prepared from the leaves of Rhus diversifolia harvested in a location where the species is common. The oleoresin was administered as a 1:50 dilution of poison oak/poison ivy urushiol in white petrolatum. Before each evaluation, the volunteers were asked to wash their hands with soap and water.

In the second study, five healthy individuals (male and female, 18 to 40 years of age, fair complexioned, skin types II to IV) with mild inflammatory acne participated after giving informed consent. The qualified volunteers had a history of acne, including a positive history to Rhus dermatitis. The lesions were examined at baseline and daily for 7 days after initiation of the treatment.

Finally, a patient (male, 63 years, Fitzpatrick skin type II) with inflammatory skin lesions in the jaw and neck area diagnosed with herpes zoster by a trained dermatologist gave his informed consent to be imaged with spectral imaging.

2.2 Instrumentation

We used a custom-made spectral imaging camera (MuSIS-HS, FORTH-Photonics, Athens, Greece) with 18 narrow-band filters (full width at half maximum of 10 nm) in the wavelength range of 400 to 970 nm and three broadband filters in the red, green, and blue spectral regions. The red, green, and blue filtered images were combined to compose a color image. The imaging detector was an 8-bit CCD camera with 1024\times768 pixels. The field of view was 16\times12 cm, resulting in a final image resolution of 0.156 mm/pixel for both the x and y axes. We used a filtered incandescent lamp equipped with a linear polarizer (model v600, Syris Scientific, Gray, Maine) as the light source. To avoid specular reflections we placed a linear polarizer in front of the camera lens with its plane orthogonal to the plane of polarization of the illumination light. We acquired a series of spectral images at a variety of wavelengths in the visible and NIR spectral range. Acquisition took approximately 5 s per image (a series of a minimum of six images required for chromophore calculations took 30 s). Each series comprised a hyperspectral image stack, a 3-D cube with one spectral and two spatial dimensions. The images in the stack were aligned using a modified algorithm based on minimization of distance in the Fourier domain.\textsuperscript{19} This step was enough to align the images within approximately 0.5 mm, at which level the skin tissue can be assumed to be homogeneous for the purposes of macroimaging. Each pixel in the hyperspectral image stack represented a remittance spectrum. Spectral images acquired using narrow-band filters contain information about the concentrations of chromophores that absorb light at the corresponding spectral bands and can therefore be qualitatively described based on absorption and scattering parameters. Note, however, that this information is not quantitative, partly due to the extensive overlap of the chromophore absorption profiles. To extract quantitative information about the concentrations of chromophores, the reflectance images must first be converted to optical density (absorbance) maps. Then spectral analysis algorithms can be applied on a pixel-by-pixel basis for the calculation of apparent concentrations of the chromophores. To this end, absorption spectra were calculated for each pixel as the negative logarithm of the ratio of image of interest to the image at 850 nm, where water, melanin, and hemoglobin absorptions are negligible. This normalization to the image at 850 nm also helps in minimizing artifacts due to contours. After these calculations, the resulting series of images constitutes the hyperspectral absorption image stack, where each pixel corresponds to an absorbance spectrum.

2.3 Erythema Maps

Erythema was evaluated based on the apparent concentrations of oxy-Hb. We calculated apparent concentrations of oxy-Hb and deoxy-Hb from the absorbance spectra after correction for melanin and scattering contributions according to a previously described algorithm.\textsuperscript{6,15,20,21} We refer to the calculated concentrations as “apparent” because they are based on light absorption curves and they are given in relative units. They are expected to be linearly related to “absolute” concentrations of hemoglobins in units of mass per volume of tissue. Due to the inhomogeneous nature of the tissue, such absolute units do not represent the reality and may be confusing. Using the absorbance hyperspectral image stack described in the previous section we calculated oxy-Hb, deoxy-Hb, melanin, and scattering values by applying the already mentioned algorithm to the absorption spectrum corresponding to each pixel. Briefly, melanin and scattering contribution were calculated as the slope and the intercept of a straight line fitted in the 630 to 730-nm region. Then these contributions were subtracted from the original absorption spectra and the corrected spectra were fitted for the contributions of oxy-Hb and deoxy-Hb.

Finally, we created distribution maps for each chromophore, in which the gray-scale intensity corresponded to the calcul-
lated chromophore values. Each chromophore map is a quantitative representation of the special distribution of the contribution to radiation absorption by the corresponding chromophore. Assuming that the contribution to light absorption is proportional to the chromophore concentration, the chromophore maps can be considered as quantitative representations of the chromophore concentrations. We considered the oxy-Hb map to represent a quantitative map of erythema involvement. To quantitate erythema we used the average gray-scale value over a region of interest defined by the size of the lesion to be measured. A neighboring area of uninvolved skin of similar size was chosen for reference. The apparent concentration of oxy-Hb corresponding to neighboring uninvolved skin sites was subtracted.

2.4 Edema Maps

NIR radiation is absorbed by water with a local maximum at 970 nm. Water absorbance maps were calculated as the negative logarithm of the pixel intensity values of the image at 970 nm, where water has an absorption maximum, normalized to the corresponding pixel intensity values of the image at 850 nm (negligible NIR absorption by water). The normalization provides a baseline for the measurement of NIR absorption and minimizes artifacts due to contours. The amount of NIR absorption at 970 nm was assumed to be proportional to water content in the tissue. The absorbance map at 970 nm can be considered as a quantitative map of water accumulation in the tissue and therefore of edema. At these wavelength range light penetrates 1.2 to 1.6 mm into the skin, and therefore the information about cutaneous edema is limited to the tissue volume corresponding to this depth. Similarly to erythema, apparent water concentration values were averaged to the corresponding pixel intensity values of the image at 850 nm (negligible NIR absorption by water). The normalization provides a baseline for the measurement of NIR absorption and minimizes artifacts due to contours. The amount of NIR absorption at 970 nm was assumed to be proportional to water content in the tissue. The absorbance map at 970 nm can be considered as a quantitative map of water accumulation in the tissue and therefore of edema. At these wavelength range light penetrates 1.2 to 1.6 mm into the skin, and therefore the information about cutaneous edema is limited to the tissue volume corresponding to this depth. Similarly to erythema, apparent water concentration values were averaged.
over a region of interest based on the lesion size. The apparent water concentration corresponding to neighboring uninvolved skin sites was subtracted.

3 Results

3.1 Allergic Dermatitis (Poison Ivy)
The volar forearms of five volunteers with positive history to Rhus dermatitis were induced by urushiol for 2 h under occlusion, and the reaction was followed for 1 week. On day 1 only, two subjects developed a mild rash, while on day 3 all of the volunteers had developed a reaction including erythema and edema. Erythema was intense and limited to the area where urushiol came in contact with the skin. Edema was mild and present as perifollicular swellings within the area of induction. On day 7, the reactions persisted on all volunteers as perifollicular swellings within the area of urushiol contact. Edema was apparent concentration values calculated from the spectral analysis is expressed in oxy-hemoglobin values. Data are given as mean ±1 standard deviation. The correlation coefficient of the two methods is R=0.979 (data shown in Fig. 3).

3.2 Acne
Spectral imaging was used to evaluate the intensity and extent of erythema and edema in acne lesions. The lesions were monitored over a period of 1 week. Similarly to the poison ivy study, erythema maps were constructed based on the oxy-Hb apparent concentration values calculated from the spectral images.

![Fig. 2 Apparent concentration maps for the skin chromophores demonstrate the intensity and spatial distribution of erythema and edema. The maps shown here were calculated from the images shown in Fig. 1. The urushiol-induced inflammatory reaction demonstrates significantly elevated concentration of oxy-Hb compared to surrounding skin. Deoxy-Hb concentration is elevated moderately and the increased water concentration relates to edema formation. For comparison, the melanin map is also shown. Inflammation has not affected the local melanin concentration. In all the maps the scale is linear. The maximum gray-scale value (255) corresponds to apparent concentration of 10 for oxy-Hb, deoxy-Hb, and melanin, and 0.5 for water. The minimum gray-scale intensity (0) corresponds to zero concentration for all the maps.](image)

![Fig. 3 Erythema values obtained by spectral image analysis correlate with the clinical evaluation. Clinical evaluation of erythema was based on five-scale grading (0 to 4) with 0 being no detectable reaction and 4 being the most severe reaction. Erythema calculated from spectral analysis is expressed in oxy-hemoglobin values. Data are given as mean ±1 standard deviation. The correlation coefficient of the two methods is R=0.979.](image)
the evolution of an acne inflammatory lesion over time. Lesion size are relevant parameters that can be used to monitor the lesion. This procedure gives an estimate of the lesion area. The percentage change in lesion area from baseline over time shows only the ones that are at the stage of papules. Interestingly, these are the lesions that are most painful due to activation of the pain receptors by the local buildup of pressure.

The gray-level intensity of the erythema map has a linear relationship with the apparent concentration of oxy-Hb. This means that regions of interest (ROIs) can be drawn around each lesion and the apparent amount of oxy-Hb can be quantified and monitored over time. In this procedure, we used a circular ROI centered at the geometrical center of the lesion. The same ROI is used on the same lesion for all the images in the subsequent time points. The difference of the lesional erythema intensity from the erythema level of neighboring uninvolved skin can be recorded and plotted over time [Fig. 5(a)]. Another way of analysis is to threshold the erythema maps not only give a visual aid of the distribution of erythema and edema, but they can also be used to calculate relevant parameters of disease progression, such as lesion count, lesion size, and erythema intensity.

The first model of skin inflammation we examined was rhus dermatitis. This condition is an allergic skin reaction caused by the Rhus (toxicodendron) genus of plants. Members of this genus are poison ivy, poison oak, and poison sumac. Plants in the Rhus genus produce an irritant pentadecylcatechol called urushiol in their stems, roots, canals, and skin of the fruits. When urushiol comes in contact with the skin, it causes an allergic reaction, commonly known as poison ivy rash.

In this paper, we demonstrated the applicability of spectral imaging to document skin inflammatory reactions. We provided examples of skin inflammation due to allergic contact dermatitis, inflammatory acne, and viral infection. In all cases, we demonstrated that using spectral imaging we can construct quantitative maps of apparent concentration of oxy-Hb and water corresponding to maps of erythema and edema. These maps not only give a visual aid of the distribution of erythema and edema, but they can also be used to calculate relevant parameters of disease progression, such as lesion count, lesion size, and erythema intensity.

3.3 Herpes Zoster
Spectral imaging was used to document the inflammatory reaction on a patient diagnosed with herpes zoster on the cheek and jaw area (Fig. 6). The oxy-Hb apparent concentration map provides better contrast than the conventional RGB image for erythema evaluation. Individual papules are easily detected as well as their agglomerations. Deoxy-Hb is slightly elevated in the inflamed area, though not to the levels of oxy-Hb, indicating the high demand for oxygen compared to surrounding tissue. Accumulation of extracellular fluid in the edematous areas can be demonstrated in the water apparent concentration map. These reactions are difficult or impossible to be identified in the visible image.

4 Discussion
In this paper, we demonstrated the applicability of spectral imaging to document skin inflammatory reactions. We provided examples of skin inflammation due to allergic contact dermatitis, inflammatory acne, and viral infection. In all cases, we demonstrated that using spectral imaging we can construct quantitative maps of apparent concentration of oxy-Hb and water corresponding to maps of erythema and edema. These maps not only give a visual aid of the distribution of erythema and edema, but they can also be used to calculate relevant parameters of disease progression, such as lesion count, lesion size, and erythema intensity.

Herpes zoster is a viral infection acquired at childhood. The virus lays dormant in the neurons until it is triggered in adulthood by yet unknown factors, though usually associated to stress. The manifestation of the disease is cutaneous inflammation that includes erythema, edema, and often severe pain.

In all cases of skin inflammation just mentioned, inflammatory cytokines and growth factors released from keratinocytes and other cells in the skin induce a chemical cascade that results in relaxation of the arteriolar smooth muscle, which eventually leads to vasodilation and increased vascular permeability. Vasodilation of capillaries in the papillary dermis leads to increased blood flow and increased local concentr-
tration of blood. In this way, more oxygenated blood reaches the inflamed tissue. The strong blue-green absorption of hemoglobin gives the familiar red color (erythema) of the inflamed skin. In parallel, in some cases the increased vascular permeability leads to augmented interstitial fluid pressures. The onset of edema is the result of these osmotic pressures exceeding the lymphatic edema safety factors.22

Several papers have been published describing methods for measuring cutaneous erythema including colorimetric23–25 or spectrophotometric methods.5,8,21 In contrast to erythema, there are very few reports where spectroscopy was used to document the edema reaction.18 All these methods are limited to the fact that the area measured is defined by the size of the probe. Furthermore, these methods require for the probe to come in contact with the skin, so that care must be taken to avoid cross-contamination of the measured skin areas.

In contrast, spectral imaging is a noncontact method in which the observed area is defined by the field of view of the objective and the resolution of the camera. Thus, it can be focused on a single lesion or extended to the whole face or whole body, as needed.

Spectral imaging in the visible and the NIR range has been used or the study of blood oxygenation level as an indicator of tissue survival in burns26 or grafts.27 It has also been proposed for monitoring edema following histamine iontophoresis.15 We recently reported the use of spectral imaging to document cutaneous edema following histamine iontophoresis.15

In this paper, we demonstrated in vivo functional imaging of cutaneous inflammatory reactions using characteristic absorption bands of water and hemoglobin. Apparent concentration maps of oxy-Hb and water were constructed that represent quantitative visualizations of the intensity and extent of erythema and edema correspondingly. These maps can be used to extract relevant information for the assessment of the severity or to monitor the evolution of a cutaneous inflammatory reaction. The portability of the method presented here enables it to easily be adapted in a clinical or dermatology practice setting. The fact that erythema and edema are the primary clinical expressions of tissue inflammation indicates that the present method may be exploited in a wide range of medical applications following the appropriate modifications, for example, used with a catheter to document tissue inflammation during surgery.

Acknowledgments

The authors would like to acknowledge the contributions of Dr. Kays Keidbey of KGL Inc. and Laura Magee of Johnson & Johnson CPPW for the poison ivy study and of Jeanette Chantalat of Johnson & Johnson CPPW for her contribution to the acne study. The authors would like to acknowledge Prof. Costas Balas of FORTH-Photonics for the development of the hyperspectral camera.

References
