Non-Invasive Thickness Measurement of Stratum Corneum by Light Scattering Spectroscopy†¹

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We describe a light scattering methodology that could facilitate a non-invasive, in vivo measurement of the thickness of stratum corneum in skin, as well as other physical observables. Namely, the in vivo method could well be based on the detailed observations of the scattered photons emitted at the surface of skin, as a laser beam focused on the surface propagates within. Preliminary in vitro investigations using this methodology on pig skin indicate that it is possible to distinguish the boundary between the stratum corneum (SC) and the Malphigian layer (ML), via the measured intensity of photons emitted at the surface but along the expected trajectory of the laser beam within the skin. The thickness of the SC could then be calculated from the measured distance between the skin surface and the SC/ML boundary, if also the angle of trajectory of the transmitted light is known (Snell’s law). Since this angle is dependent on the unknown refractive index of SC, further measurements at different incident angles were necessary to determine the SC thickness and refractive index. Based on the results of our preliminary studies, it is reasonable to predict that light scattering methodologies could be useful for cosmetic industry.

Introduction

The field of cosmetic science and technology exists to define and perpetuate the concept of good, healthy skin. Because this field of science is of great interest in commercial applications, we persist to reduce the somewhat complex physiological functions of the body’s largest organ to identifiable parameters that can be readily quantitated and exploited. For example, a great body of work has already established several physiological essentials necessary for the continuous development and maintenance of healthy skin for healthy individuals. That is, it is well-understood that good, healthy looking skin requires the presence of intercellular lipids (ICL) of a specific composition¹ as well as the natural moisturizing factor (NMF)². To quantify as well as convey the concept that the lack of ICL’s and NMF results in a dry and scaly stratum corneum simply, however, requires the establishment of a readily usable set of physical measurables as criteria. One established set of criteria for demonstrating the achievement of good healthy looking skin are impedance measurements. But because these measurements alone are often incomplete, we also rely on visual verification by CCD based image analyses of the skin surface to confirm the veracity of our impedance results.

Image analyses of skin has been one of the primary tools used to promote as well as assess...
many concepts that involve the achievement of good healthy skin. In fact, many of the tools that are used to assess the efficacy of cosmetic agents are based on the interaction of light with skin; for example, anti-pigmentation/UV protection agents\(^3\), anti-wrinkling agents\(^4\), blood flow rate\(^5\), etc. Although there exists substantiative amount of skin imaging data that correlates well the efficacy of our products with their intended uses, there is yet a fundamental lack of understanding of the nature of light interaction with the skin, of particular interest being the epidermal layer. Not only will a more detailed understanding of this interaction facilitate an appropriate analyses of existing data, it will also lead to newer technological applications that could better enable us to classify the conditions that define healthy looking skin. For example, it may well be possible to quantify the degree of scaliness of dry skin. Moreover, it may also be possible to use modified versions of existing imaging technology for assisting in the diagnosis of severe skin disorders much more accurately.

In addition to developing a better understanding for the light interaction with skin to define healthy looking skin, we are also interested in developing a model to define good feeling which relies on the determination of the physical characteristics of the skin, principally the stratum corneum thickness and elasticity.\(^6\) It has been proposed that knowledge of the behavior of these two parameters are essential for new developments in the design, preparation and testing of cosmetic products that achieve healthy, good looking and feeling skin. Hence we now report preliminary data that are in part the development of technology which makes use of laser light scattering methodologies to determine the physical characteristics of the stratum corneum (SC). In particular, this manuscript will demonstrate the usefulness of light scattering methodologies in determining the thickness of SC.

### Materials and Methods

#### I. Experimental Materials

**Model stratum corneum sheet:** The preparation of a thin film of layered horny cells was accomplished following procedures modified from methods developed by Smith and colleagues\(^7\). In our study we prepared the model stratum corneum from fresh pig skin purchased from a local supplier, as follows: 1) we removed unwanted hair from small sections of pig skin with a shaver, 2) small sections were cut and boiled in distilled water at 60°C for 1 min., 3) the epidermal and possibly the dermal layers were peeled off the skin with tweezers and then stirred in a trypsinated 20 mM phosphate buffered solution for 1 hr., which separates the dermis from the epidermis, 4) the dermis layer was then removed, leaving sheets of SC from pig skin, 5) the sheets were washed with water and dried, and were then cut into smaller pieces and homogenized suspension of fragmented SC was then prepared in a chloroform: methanol: water solution, 6) a thin film of this suspension confined in a Teflon O-ring was allowed to dry, thereby forming the model SC sheet. The dried model SC sheet was soaked in water prior to use in our measurements. To prevent from drying out during the course of our measurements, the water saturated model SC sheet was covered with a microscope slip.

**Pig skin:** Fresh pig skin was purchased from a local supplier. The skin was first washed, cut into manageable pieces and the hair manually removed to permit better reproducibility of data, as the unwanted dirt and hair interferes with light scattering results. Prior to use, the pig skin was not subjected to further modifications.

#### II. Experimental Methods

A schematic diagram of the light scattering apparatus used to measure the thickness and refractive index properties of the above materials is
shown in Fig.-1. The design of the apparatus is based on the microscope laser light scattering spectroscopy technology implemented by Dr. I. Nishio et al. at MIT for studies involving the dynamics of living cells. The present equipment consists of a 30 milli-watt laser whose emitted light is focused into an optical fiber. The optical fiber is a light guide; light exiting the optical fiber is focused with the aid of a GRIN lens onto a sample positioned at the focal plane of the microscope. That is, the sample surface, the focal point of the laser beam and the focal point of the microscope objective are coincident on the same plane. The back scattered light at an angle $\theta$ collected by the microscope objective is collimated into a microscope eyepiece that has a "pick-up" optical fiber which can translate parallel to the focal plane of the sample surface. The back scattered light collected by the optical fiber within the eyepiece is then guided to a photomultiplier tube (PMT) via an optical fiber bundle. The PMT converts the collected photons into electrons with a gain of $\approx 10^6$. The current exiting the PMT is then amplified by a factor of $\approx 10^6$ and forwarded to a digital multimeter. The photon flux is thus represented as voltage in the digital multimeter that is read by a computer via GPIB interface.

The scattered light sampling volume using the above system configuration which makes use of a 32x microscope objective with a variable iris to limit reflections is $\approx 8 \mu m^3$, whereas the incident focused laser beam has a measured diameter of $\approx 40 \mu m$ with a depth focus of $\approx 40 \mu m$. Once the intensity of scattered light emitted at a point on the surface of the scattering medium but on the focal plane of the microscope is averaged for approximately 15 sec, the translating fiber optic within the eyepiece is moved to another position on the sample. That is, while maintaining the focal positions of both the incident laser and the microscope objective on the surface of pig skin, we translated the "pick-up" optical fiber in approximately $1.5 \pm 0.2 \mu m$ increments along the microscope objective focal plane following the expected trajectory of the laser beam as it propagates through the sample. Fig.-2 displays a typical profile of the laser beam trajectory in two types of samples; photographs taken at 40X magnification. As can be readily observed, the trajectory of the laser beam is quite simple to follow. All experiments following the above procedures were then performed at room temperature and humidity.

**Results**

There are several possible ways to measure the thickness of a medium based on the behavior of
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light as it is scattered and/or reflected while propagating through the medium, and principles of optics\(^9\). Our method is based on the measurement of surface photons along the axis of light propagation. Data obtained, by using the above methodology for the light scattering system, for a microscope glass cover slip, a model SC sheet and SC of fresh pig skin are shown in Fig.-3, where the obtained averaged intensity and its measured standard deviation are plotted as a function of each translational fiber optic position. The solid lines are a Gaussian fit to the data, however, the results are intended to be a guide to the eye and have no theoretical significance at present.

Independent of the type of medium under investigation, the significant feature of the data is that there exists periodic maxima as the position of the translating pick-up optical fiber is varied. Particularly for the case of the model SC sheet and the SC of pig skin, the observed maxima are well within the \(1/e^2\) Gaussian diameter of the focused laser beam (see also Discussion section). Lacking appropriate theoretical or experimental models that would enable precise interpretation of our data, however, we simply assumed that the distance between the periodic maxima (see Fig.-3) represent interfacial boundaries where scattered/ reflected light is expected to be increased\(^9\). Namely, the first maxima represent the first interface between the air and the medium (or SC), whereas the secondary maxima represent the boundary between the medium and the air (or in the case of pig skin the malphigian layer). Hence imposing this simple assumption and invoking Snell’s law, it should be possible to determine the thickness of the medium provided that its refractive index is well-known (see Fig.-4). Of the three different media, only the refractive index of glass is known, \(n_2=1.55\). Since the refractive indices of the model SC sheet and the SC of pig skin are not quantities that have been previously measured\(^{10,11}\), it was then necessary to measure the changes in the distances between the

![Fig.-2 Displays the propagation properties of a laser beam in two different media whose light scattering properties are dissimilar. In (A) the laser beam is traversing from left to right through a sample solution containing latex particles that are smaller than the wavelength of light. In (B) the focused laser beam is shown traversing through pig skin. The numbered black dots represent figuretively the positions of the optical pick-up fiber. As evidenced, the laser focal spot becomes more disperse when passing through the pig skin due to multiple-scattering effects.](image)
periodic maxima as the angle of incidence of the laser light was varied, in order to determine the thickness of these materials (also see Fig.-4).

**Fig.-3** Averaged intensity data as a function of surface position are displayed for each sample investigated at an incident angle of 49°. (A) Glass cover slip, (B) Model SC sheet and (C) SC of pig skin.

**Fig.-4** A simplistic view of the propagation of light within pig skin is presented in terms of Snell’s law, where $\phi_i$, $\phi_r$, and $\phi_t$ are the incident, reflective and transmitted angles, respectively. The equation used to determine the thickness ($Z$) is also shown, where $n_1$ and $n_2$ are the refractive indices of air and refractive medium, respectively. The equation derived by using simple geometric relation shown above described well the dependence of the changes in the observed distance between maxima as a function of the incident angle.

**Fig.-5, Fig.-6 and Fig.-7** exhibit the changes in the distances between observed maxima for six different laser incidence angles utilized for the three different media under investigation. As expected, the distances between the observed maxima had increased with respect to the increased incident angle of the laser beam. If these changes in the data do indeed reflect behavior as predicted by Snell’s law, then a parametric fit to the data by the simple equation shown in Fig.-4 should yield realistic values for the thickness of the medium under investigation, as well as its refractive index. As shown in Fig.-5, Fig.-6 and Fig.-7, the changes in the distances between maxima are well characterized by a Snell’s law fit to the data. The results of a two parameter fit to the data by the equation based
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Table-1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Expected</th>
<th>Measured</th>
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<tbody>
<tr>
<td></td>
<td>Refrac. Index</td>
<td>Thickness</td>
</tr>
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<td>Glass cover</td>
<td>1.55</td>
<td>98±6 μm</td>
</tr>
<tr>
<td>SC in sheet</td>
<td>1.55[10,11]</td>
<td>30±5 μm</td>
</tr>
<tr>
<td>SC in skin</td>
<td>1.55[10,11]</td>
<td>12±1 μm</td>
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</table>

Discussion

We are interested in developing technologies which would facilitate the production and promotion of new cosmetics. One facet of our developing technology relies on the interaction of light with
biological tissue, which had already received a great deal of interest especially in the field of diagnostic and therapeutic medicine. One of several significant contributions of this field has been the description of the propagation of light within the skin. For instance, there are several existing theories that predict the behavior of photons propagating in disperse media, like the skin\textsuperscript{12},\textsuperscript{13}. Of the many results obtained from their investigations, the following may be applicable towards the interpretations of data presented herein: 1) The stratum corneum is a turbid medium where multiple scattering persists. 2) Due to this condition, light propagating within the SC is broadened by photon diffusion. 3) As light propagates within the skin, reflectance from the various layers is effected by the changes in the scattering geometry of the particles residing within each layer as well as the particle density. And 4) visible light is forward scattered\textsuperscript{14}, however, the depth which the photons had traveled prior to being re-emitted at a point on the surface is also dependent on the photon diffusive properties of the skin. Of these four basic results based on both the theoretical as well as experimental investigations, the first three are directly applicable to the present study.

Consider now the propagation of laser light of visible wavelength $\lambda=632$ nm (He–Ne) within a simple solution containing particles whose diameter is smaller than $\lambda$. Within this system where there are little or no effects due to multiple scattering because the particle concentration is low, the light is scattered uniformly in all directions and the overall “shape” of the propagating laser beam is maintained, i.e. no broadening due to photon diffusion is observed. As shown in Fig.-2(A), the beam profile is maintained, i.e. the measured focal diameter of the laser beam at the $1/e^2$ limit is still 40 $\mu$m. When the laser light is focused on pig skin, however, broadening can be readily observed (see Fig.-2(B)). From the extent of broadening, which is approximately 3x the original laser focal diameter, it is possible to obtain specific characteristics of the SC\textsuperscript{15}. At present, however, we simply need to note that the trends in the data obtained for the determination of the thickness of the model SC sheet and the SC of pig skin, as shown in Fig.-3, lie within this broadened region. This result implies that the photons emitted at the surface had not penetrated a significant distance within the skin (e.g. dermis), and that the maxima observed in Fig.-3(B) and Fig.-3(C) are quite possibly due to reflections emanating from the surface or from the boundary between the SC and air or SC and Malphigian layer, respectively. We
believe that the primary and secondary maxima observed in Fig.-3(B) and Fig.-3(C) are due to reflections from the first air/SC interface and the second air/SC or SC/Malpighian layer interface, as predicted by theory\(^1\(^3\). Because, if they simply are due to reflections from the surface alone, the spacing between maxima would then be on the order of horny cell size, approximately 2–3 μm. In addition, the intensity of the reflections as well as the change in the distances between the maxima would exhibit markedly different characteristics that were not observed in our data (not shown). Moreover, the design of the apparatus is such that we observe the scattering of photons at the surface rather than reflections. Indeed, the maxima observed for the microscope glass cover slip is evidently due to reflections/scattering at the two different interfaces, because this medium is not optically dense.

Thus assuming that the changes in the distances between the maxima with respect variations in the incident angle can be represented by Snell’s law, the observed data were fitted accordingly to obtain both the thickness of the simple as well as the refractive index using the derived equation shown in Fig.-4. The results of the two parameter fit to the data yielded reasonable results for the thickness of our samples as well as its refractive index. That the thickness of the glass microscope coverslip was overestimated and its refractive index underestimated may be due to the uncertainty in the measured incident angle of the laser beam, as well as the uncertainty that both sides of the glass are not parallel. The expected thickness for the glass coverslip was determined by initially focusing on the top surface and then subsequently measuring the distance the microscope stage was translated to focus on the bottom surface.

The expected thickness for both the model SC sheet and the SC of the pig skin was determined by microscopic examination of a frozen cross-section (data not shown). The thickness obtained for the model SC sheet as well as the SC of pig skin were in remarkable agreement. The refractive indices were, however, somewhat low. The expected refractive index for both these media was reported as 1.55\(^{10,11}\), which is quite unrealistic. The refractive index should, in general, reflect nature of the medium which we believe to be water or cosmetics when applied. This is a subject of further investigations.

We believe, however, that data obtained by our methodology do indeed indicate that the thickness of the SC can be estimated by invoking Snell’s law, and thereby the refractive index can also be directly measured without having to invoke assumptions.

Since we are attempting to establish new technologies for the cosmetic industry based on the principles of light scattering from within skin, it is important to maximize the applicability of the system as much as possible. When this method is applied for the in vivo studies of the thickness of the human SC, the collection times will have to be much shorter (order of several seconds). At the present time the collection times are much too long to observe the changes in the thickness of the SC when treated by cosmetics, such as lotions. Technology that are currently available can be readily exploited to achieve our goals.

**Conclusions**

We have made several important preliminary determinations on the application of a light scattering methodology to determine some physical characteristics of the skin. Namely, it was possible to determine the thickness of SC by observing the scattered light intensities emitted at the skin surface from scattering within. Using our developed methodology, it was possible to distinguish the boundary between the SC and the Malphigian layer. Thus we were able to determine the SC thickness as well as its refractive index. We also believe that this methodology could be adapted to perform in vivo measurements to assist in the
development and assessment of new cosmetic agents.

Acknowledgements

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References


6) S. Gorti, Unpublished Investigations


