Can reduced scattering coefficients obtained using inversion procedures lead to unsound inferences about the optical factors contributing to the appearance of blood vessels embedded in the skin?

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Abstract. Advances in tissue appearance modelling have allowed researchers to perform in silico (computational) evaluations of the relationship between the morphological characteristics of a given tissue and its appearance. Two recent works in this area considered multiple tissues in order to simulate the appearance of blood vessels underneath the skin. However, the authors of these works provided conflicting conclusions regarding the importance of Rayleigh scattering’s contribution to the bluish appearance of veins. Additionally, the authors of the later publication provided information that may lead readers to misinterpret the methodology and conclusions of the earlier work. In this paper, we outline the differences between the investigation approaches employed in both works, notably elaborating on the use of reduced scattering coefficients in the later work. We then highlight key aspects of the earlier work in order to correct any potential misinterpretations and analyze the distinct conclusions presented by the authors of both works.

Keywords: vein appearance; tissue optics; predictive simulation; skin spectral responses; blood spectral responses; Rayleigh scattering.

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1 Introduction

The bluish appearance of veins has long been a topic of interest for biomedical optics researchers. A couple of decades ago, Kienle et al.\(^1\) examined the main factors responsible for the bluish appearance of veins. We will now provide an overview of these factors.

First, the optical properties of the relevant tissues must be considered. Optical scattering in the skin favours the blue region of the visible spectrum. As a result, much of the blue light that enters the skin is remitted before it can reach a subcutaneous vein. Naturally, blue light is backscattered by the skin regardless of what lies beneath it. Accordingly, we must also attribute the bluish appearance to the strong absorption of longer-wavelength light by the deoxygenated blood flowing through the subcutaneous vein.

In addition to the above considerations, aspects of human perception must also be taken into account when discussing the bluish appearance of veins. Specifically, Kienle et al.\(^1\) proposed that a greater remission of blue light than red light by the tissues is not necessary for a vein to be perceived as bluish. Rather, the ratio of the difference between blue and red remissions measured above the vein to the respective difference measured above the surrounding tissue determines whether a vein appears bluish. This perceptual phenomenon can be explained by Retinex theory.\(^2\)

While the explanation provided by Kienle et al.\(^1\) for the bluish appearance of veins has been generally accepted by the scientific community, it is still possible to deepen our understanding of this phenomenon. In particular, the skin is a complex organ that can be anatomically subdivided into several layers, each of which is composed of distinct constituent materials and heterogeneous structures. Even though it has been determined that scattering in the skin plays a key role in the bluish appearance of veins, it is still not fully understood how each of the individual heterogeneous structures in the skin affect its overall optical properties.
In a recent publication by Van Leeuwen and Baranoski, they presented a framework for simulating subcutaneous vessel appearance and evaluated the potential contribution of optical scattering by collagen and reticulin fibrils in the papillary dermis to the overall appearance of subcutaneous veins by performing \textit{in silico} (computational) experiments. Given the radius of these fibrils, their scattering behaviour was modelled using the Rayleigh scattering theory. Van Leeuwen and Baranoski stated that their results indicate that Rayleigh scattering caused by collagen fibrils in the papillary dermis can play a pivotal role in the bluish appearance of veins.

Subsequently, Zoller and Kienle independently simulated the appearance of subcutaneous vessels and advanced the previous work of Kienle et al. by visually demonstrating the effect of depth, radius and oxygenation on modelled subcutaneous vessel appearance. They also presented results obtained by varying the reduced scattering coefficient employed in their simulations in order to evaluate how the scattering properties of the skin can affect the appearance of subcutaneous veins. They claimed that these results demonstrate that Rayleigh scattering does not have a major influence on the bluish appearance of veins. They also presented information that may lead readers to misinterpret the methodology and conclusions of Van Leeuwen and Baranoski.

The primary goal of this paper is to address the conflicting conclusions presented by Van Leeuwen and Baranoski and by Zoller and Kienle in regard to the contribution of Rayleigh scattering to the bluish appearance of veins. In order to discuss their respective conclusions, we will first outline the relevant differences between the investigation approaches employed by these works in Section 2. In Section 3, we will then clarify key aspects of the work by Van Leeuwen and Baranoski in order to correct any potential misinterpretations of this work resulting from the information provided by Zoller and Kienle. Next, we will discuss the conclusions presented by both of these publications in Section 4. In particular, we will explain why the results presented in the paper by Zoller and Kienle do not weaken the conclusions presented by Van Leeuwen and Baranoski. We will achieve this by systematically examining their investigation approach to show why the results presented in their publication are not sufficient to refute the proposal that Rayleigh scattering has a major influence on the bluish appearance of veins. Finally, in Section 5, we will provide some concluding remarks.

2 Background

In the following subsections, we will outline the relevant differences between the investigation approaches employed by Van Leeuwen and Baranoski and by Zoller and Kienle. We emphasize that our goal is to focus on differences that are relevant to the discussion in this paper, not to fully describe both investigation approaches. For full descriptions of these approaches, please refer to the original publications and supplementary papers describing the first-principles models of light interaction for skin (HyLIoS) and blood (CLBlood) employed by Van Leeuwen and Baranoski.

2.1 Overview of Van Leeuwen and Baranoski’s Modelling Approach

In the paper by Van Leeuwen and Baranoski, they presented a framework for simulating vein appearance that employed previously published light-interaction models for skin (HyLIoS) and blood (CLBlood). Both of these models employ stochastic techniques based on Monte Carlo methods in their light transport simulations. Furthermore, the development of these models followed a first-principles approach. In particular, the optical processes (light absorption and scattering) within a given tissue are simulated by directly considering its constituent materials and heterogeneous structures. For example, the absorption of light within the reticular dermis is directly dependent on the molar extinction coefficient and concentration
Van Leeuwen and Baranoski\textsuperscript{3} employed a multi-layer representation of skin with a vein included beneath it, as depicted in Figure 1. Specifically, the skin was subdivided into stratum corneum, epidermal layers (stratum granulosum, stratum spinosum and stratum basale) and dermal layers (papillary dermis and reticular dermis). The vein was composed of two outer layers representing the vein wall and an inner layer representing flowing blood.

In Section 2.1.1, we will provide a high-level description of the heterogeneous structures considered in the formulation of the employed skin (HyLioS\textsuperscript{7}) and blood (CLBlood\textsuperscript{3,11}) models. Then, in Section 2.1.2, we will fully describe the simulated light-scattering behaviour of the dermis in HyLioS.\textsuperscript{7} We refer the reader to previous publications for further information regarding the investigation approach employed by Van Leeuwen and Baranoski.\textsuperscript{3,7,11,12}
2.1.1 Heterogeneous Structures in the Epidermis and Vein

HyLIoS\textsuperscript{7} and CLBlood\textsuperscript{11,12} both account for large-scale heterogeneous structures by generating them probabilistically, on the fly, along the path of the light. CLBlood generates individual red blood cells on the fly as light traverses the plasma within the flowing blood layer (Figure 2). Similarly, HyLIoS generates individual melanosomes and melanosome complexes on the fly as light traverses the epidermis. During the probabilistic generation of these structures, their abundance and orientation are taken into account. Other aspects such as size, shape and composition are also considered as light interacts with these structures. For further details, the reader may refer to previous publications describing these models.\textsuperscript{7,11,12}

2.1.2 Scattering in the Dermis

The HyLIoS model\textsuperscript{7} employs an algorithmic formulation to simulate optical scattering events in the dermal tissues. Within this formulation, the scattering caused by collagen fibrils in the papillary dermis is accounted for by using the Rayleigh scattering coefficient:\textsuperscript{7,9}

\[ \mu_s^R(\lambda) = \frac{32\pi^4 r^3 v_f \left( \eta^2 - 1 \right)^2}{\lambda^4 \left( \eta^2 + 1 \right)^2}, \]

where \( v_f \) represents the volume fraction of the papillary dermis occupied by fibrils and \( r \) represents the radius of the fibrils. The variable \( \eta \) is the ratio between the refractive index of the fibrils (\( \eta_f \)) and the refractive index of the papillary dermis (\( \eta_{pd} \)). The direction that light is scattered by the fibrils during a scattering event follows the Rayleigh scattering distribution which can be described by the following phase function:\textsuperscript{9}

\[ P(\theta) = \frac{3}{4} \left( 1 + \cos^2 \theta \right). \]

During a scattering event, the polar perturbation angle is determined using a rejection sampling algorithm which was derived using the above phase function.\textsuperscript{7} The azimuthal perturbation angle is randomly sampled from a uniform distribution. Since the employed attenuation coefficient (Equation (1)) describes a bulk scattering behaviour, the contribution of the fibrils is only taken into account once per ray traversal through the papillary dermis.

Due to the complexity of the heterogeneous structures in the reticular dermis, HyLIoS accounts for scattering in this layer by applying a diffuse perturbation to any light that reaches the bottom of it. This modelling decision corresponds to the observation that red light transmitted by the dermis is diffusely distributed.\textsuperscript{13} Since shorter wavelengths in the visible spectrum are more strongly scattered by the dermis,\textsuperscript{14} we can infer that the light transmitted by the dermis will be diffuse across the visible spectrum.

2.2 Overview of Zoller and Kienle’s Modelling Approach

Zoller and Kienle\textsuperscript{10} also performed their optical simulations using stochastic techniques based on Monte Carlo methods. In their simulations, the skin was represented by an epidermal layer and a semi-infinite dermal layer, while the vein was represented by a cylinder residing within the dermis (Figure 3). In the remainder of this section, we will provide a high-level description of the approach used by Zoller and Kienle\textsuperscript{10} to simulate optical scattering. For additional information regarding their investigation approach, please refer to their publication.\textsuperscript{10}
In the simulations of Zoller and Kienle, the scattering properties of the epidermis, dermis and vein were determined by a reduced scattering coefficient. The reduced scattering coefficient of a tissue represents the amount of light scattered out of the collimated portion of the radiance and into the diffuse portion of the radiance as light traverses the tissue. In their simulations, Zoller and Kienle employed several reduced scattering coefficient equations that were obtained from the scientific literature. They also employed an additional reduced scattering coefficient that they constructed themselves. These reduced scattering coefficients are presented below:

$$\mu'_s = \left[ 14.74 \lambda^{-0.22} + 2.2 \times 10^{11} \lambda^{-4} \right] \text{mm}^{-1} \quad (3)$$
$$\mu'_s = \left[ 2 \times 10^4 \lambda^{-1.5} + 2.2 \times 10^{11} \lambda^{-4} \right] \text{mm}^{-1} \quad (4)$$
$$\mu'_s = 5.5 \left( \frac{\lambda}{500 \text{ nm}} \right)^{-1.5} \text{mm}^{-1} \quad (5)$$
$$\mu'_s = 5.5 \left( \frac{\lambda}{500 \text{ nm}} \right)^{-4} \text{mm}^{-1} \quad (6)$$

where $\lambda$ is the wavelength of light. Equations (3) and (4) were formulated by Bashkatov et al. and Jacques, respectively, and were designed to explicitly encapsulate both Mie scattering (using the $\lambda^{-0.22}$ and $\lambda^{1.5}$ term, respectively) and Rayleigh scattering (using the $\lambda^{-4}$ term) contributions. Equation (5) was formulated by Verdel et al. who did not explicitly target any particular type of scattering and, instead, encapsulated all scattering contributions in a single term. Equations (3) – (5) were all derived by applying inversion procedures and data-fitting techniques to measured data. Equation (6) was constructed by Zoller and Kienle to represent scattering that solely follows the Rayleigh theory. In particular, the spectral dependency of this reduced scattering coefficient corresponds to the Rayleigh theory ($\lambda^{-4}$) and its magnitude was chosen such that it matches Equation (5) at 500 nm.

In their publication, Zoller and Kienle simulated the appearance of a vein beneath the skin considering...
each of the presented reduced scattering coefficients (Equations (3)–(6)). Specifically, for a given simulation, the respective value of \( \mu'_s \) was employed as the reduced scattering coefficient for the dermis and vein, and \( 2\mu'_s \) was employed for the epidermis.

## 3 Clarifications

Before discussing the conclusions presented by Van Leeuwen and Baranoski\(^3\) and by Zoller and Kienle,\(^10\) we will address some ideas presented in the latter publication\(^10\) to mitigate potential confusion regarding the work of Van Leeuwen and Baranoski.\(^3\)

### 3.1 Reduced Scattering Coefficient

Recall from Section 2.2 that the reduced scattering coefficient presented in Equation (6) was constructed by Zoller and Kienle\(^10\) to represent scattering that solely follows the Rayleigh theory. In their publication,\(^10\) they labelled Equation (6) as “Leeuwen18", implying that this equation for \( \mu'_s \) corresponds to the scattering behaviour in the simulations performed by Van Leeuwen and Baranoski.\(^3\) However, as we saw in Section 2.1, Van Leeuwen and Baranoski\(^3\) only employed the Rayleigh theory in the papillary dermis, whereas Zoller and Kienle\(^10\) employed \( \mu'_s \) as the reduced scattering coefficient for the dermis and vein, and \( 2\mu'_s \) as the reduced scattering coefficient for the epidermis. Furthermore, Rayleigh scattering was not the only kind of optical scattering considered by Van Leeuwen and Baranoski.\(^3\) As mentioned in Section 2.1, they also considered scattering by individual melanosomes and melanosome complexes in the epidermis as well as scattering by individual red blood cells in the blood flowing through the vein. They also accounted for scattering in the reticular dermis by diffusing light that passes through it. Equation (6), presented by Zoller and Kienle\(^10\) in their work, does not account for any of these optical scattering behaviours. Accordingly, the reduced scattering coefficient presented in Equation (6) and the corresponding vein appearance result produced using Equation (6), also presented by Zoller and Kienle\(^10\) with the label “Leeuwen18”, are not representative of the work by Van Leeuwen and Baranoski.\(^3\)

### 3.2 RGB Value Comparison

In the publication of Zoller and Kienle,\(^10\) they wrote, “[It] was stated [by Van Leeuwen and Baranoski] that the \( \lambda^{-4} \) dependency of \( \mu'_s (\lambda) \) is the decisive reason that leads to a significantly higher reflection for the blue channel B over the red channel R above the vessel.” Note that the reflection for the blue and red channels refers to colour channel values in the RGB (Red, Green, Blue) colour space. As mentioned in Section 1, a greater remission of blue light than red light by the tissues does not determine whether a vein will appear bluish. Rather, the ratio of the difference between blue and red remission measured above the vein to the respective difference measured above the surrounding tissue determines whether a vein is perceived as bluish. However, to our knowledge, no psychological study has been performed to precisely quantify when a human would perceive and classify a given vein appearance as “bluish”. For this reason, any present investigation regarding the bluish appearance of the veins cannot fully rely on quantitative metrics (e.g., RGB values). Instead, visual representations of a vein’s appearance (e.g., appearance swatches), generated from chromatic values that were computed considering environmental (e.g., lighting) and perceptual (e.g., photoreceptor sensitivity) aspects, should be employed. Furthermore, these representations should include the appearance of the skin above the vein as well as the appearance of the surrounding tissue so that the reader’s visual system will implicitly account for the perceptual phenomenon mentioned earlier in this section.

In their publication, Van Leeuwen and Baranoski\(^3\) acknowledged the subjective nature of classifying a vein’s appearance as bluish, and presented visual results in the form of appearance swatches accordingly.
They also presented the RGB triplet values that were employed to generate their appearance swatches and made the following statement in their paper: “The colors depicted in these swatches generally match our expectations for the appearance of skin with and without a subcutaneous vein. Accordingly, the RGB color triplets used to generate these swatches ... quantitatively show that the colors elicited by the skin specimen with a subcutaneous vein have a blue component that exceeds in magnitude its red and green counterparts.”

This statement, however, was not intended† to imply that the blue channel having a higher value than the red and green channels is the determining factor for whether a vein appears bluish. As discussed previously in this section, other factors should be taken into account. To address the earlier quote by Zoller and Kienle,† we remark that the conclusions made by Van Leeuwen and Baranoski regarding the pivotal contribution of Rayleigh scattering were made with respect to to the bluish appearance of veins and the corresponding visual results presented in their publication, not the corresponding RGB values.

4 Discussion

In this section, we will discuss conclusions presented by Van Leeuwen and Baranoski and by Zoller and Kienle regarding the contribution of Rayleigh scattering to the bluish appearance of veins. In their paper, Van Leeuwen and Baranoski supported the hypothesis that Rayleigh scattering can play a pivotal role in the bluish appearance of veins. Alternatively, Zoller and Kienle disputed that Rayleigh scattering does not have a major influence on the bluish appearance of veins. We will discuss the conclusions presented in both papers and determine whether the results presented by Zoller and Kienle are sufficient to eliminate the possibility that Rayleigh scattering contributes significantly to the bluish appearance of veins.

The conclusions presented by Van Leeuwen and Baranoski were summarized as follows: “The in silico experimental results presented in this paper support the hypothesis that Rayleigh scattering caused by collagen fibrils found in the papillary dermis can play a pivotal role in the bluish appearance of veins.” The results that this quote refers to showed that, by considering Rayleigh scattering caused by collagen fibrils in the papillary dermis, their simulations were able to reproduce the bluish appearance of veins. Alternatively, when considering all other aspects of the models employed in their vein appearance simulations (e.g., light absorption by various pigments, optical scattering by individual melanosomes, etc.) without Rayleigh scattering by the collagen fibrils in the papillary dermis, their in silico experiments did not produce a bluish appearance for the vein. What these results indicate is that the collagen fibrils in the papillary dermis, when considered alongside the other aspects of the employed models, are theoretically sufficient to produce a bluish vein appearance.

When discussing the results of Van Leeuwen and Baranoski, we must keep in mind that the models employed in their vein appearance simulations were designed using a first-principles approach based on the current understanding of the structure of skin, and all parameter values employed in their in silico experiments were biophysically valid and consistent with measured ranges reported in the scientific literature. Furthermore, the models for skin (HyLioS) and blood (CLBlood) were both validated quantitatively and qualitatively in their original publications, and have been employed in a wide range of biomedical investigations.

In response to the work by Van Leeuwen and Baranoski, Zoller and Kienle wrote, “[Van] Leeuwen [and Baranoski] postulated that the Rayleigh scattering of skin has a major influence on the typical bluish color of veins. [It] was stated [by Van Leeuwen and Baranoski] that the $\lambda^{-4}$ dependency of $\mu'_{s} (\lambda)$ is the decisive reason that leads to a significantly higher reflection for the blue channel B over the red channel R above the vessel. However, as Figs 9(a) – 9(d) show, this is not important, because the vessel appears bluish

†Note that we know the intent of this statement because we were the authors of this paper.
in all images.” In the figure that this quote refers to, Zoller and Kienle\textsuperscript{10} presented vein appearance swatches generated using their simulation framework. The subfigures of interest corresponded to each of the reduced scattering coefficients presented in Equations (3) – (6) of this publication.

The conclusion of Zoller and Kienle\textsuperscript{10} follows from the fact that they have reproduced the bluish appearance of veins while employing a reduced scattering coefficient that does not have an explicit Rayleigh scattering term (Equation (5)). However, the form of this reduced scattering coefficient does not necessarily provide any insight into how individual heterogeneous structures may contribute to the overall scattering behaviour of a corresponding living skin specimen. To illustrate this point, let us examine the methodology that Bashkatov \textit{et al.}\textsuperscript{16} used to obtain Equation (3) and the methodology that Verdel \textit{et al.}\textsuperscript{18} used to obtain Equation (5).

Bashkatov \textit{et al.}\textsuperscript{16} applied the inverse adding-doubling method to compute reduced scattering coefficient values for a sample skin specimen at the measured wavelengths. This inversion procedure employs measured diffuse reflectance and total transmittance data for the sample skin specimen. They then used data fitting methods to formulate an equation that fit the computed reduced scattering coefficient values. During the data fitting procedure, the shape of the reduced scattering coefficient data determined the necessity to incorporate both Rayleigh scattering and Mie scattering terms.

Verdel \textit{et al.}\textsuperscript{18}, on the other hand, applied a nonlinear least squares method to perform a model-based inversion in order to find a reduced scattering coefficient for the sample skin specimen. This procedure employs measured diffuse reflectance and pulsed photothermal radiometric data for the sample skin specimen. Additionally, when computing the reduced scattering coefficient for the specimen, this approach assumes that the reduced scattering coefficient has the form:

\[ a \left( \frac{\lambda}{500 \text{ nm}} \right)^{-p}, \]

where the amplitude \( a \) and power \( p \) are values being computed by the inversion procedure.

In both of these works,\textsuperscript{16,18} the authors obtained a reduced scattering coefficient for their sample skin specimen that produced modelled data that matched the original measured data with good agreement. However, Bashkatov \textit{et al.}\textsuperscript{16} obtained a reduced scattering coefficient while employing a Rayleigh scattering term, whereas Verdel \textit{et al.}\textsuperscript{18} obtained a reduced scattering coefficient without employing a Rayleigh scattering term. This brings the question: why do both reduced scattering coefficients, although conceptually distinct, produce modelled data that agrees well with the measured data employed by the respective authors? While this could be attributed to differences between the measured specimens, it could also be attributed to the possibility that many different reduced scattering coefficients with different forms can produce modelled data that agrees well with a given set of measured data. The likelihood that the latter possibility is applicable increases if the other parameters of the given model are flexible during the corresponding inversion process, as is the case in the procedures employed by Bashkatov \textit{et al.}\textsuperscript{16} and by Verdel \textit{et al.}\textsuperscript{18}.

In fact, if we assume that these procedures are equally reliable and robust (i.e., given an arbitrary skin specimen, they can both compute a reduced scattering coefficient that produces modelled data that provides good agreement with the measured data), then we can deduce that, for at least some skin specimens, there are several reduced scattering coefficients with different forms that can produce modelled data that provides good agreement with measured data. This is because the respective procedures employed by Bashkatov \textit{et al.}\textsuperscript{16} and by Verdel \textit{et al.}\textsuperscript{18} cannot provide reduced scattering coefficients with the same form for all possible skin specimens. For example, the reduced scattering coefficient obtained by Bashkatov \textit{et al.}\textsuperscript{16} (Equation (3)) was computed for a given specimen. If provided the same specimen, the
procedure of Verdel et al. \cite{18} could not possibly produce the same reduced scattering coefficient due to the restrictions imposed on its form by their procedure (i.e., the reduced scattering coefficient is restricted to the form presented in Equation (7)).

Given the above postulation that many forms of reduced scattering coefficient could produce modelled data that provides good agreement with measured data for a given skin specimen, the form of a reduced scattering coefficient on its own is not capable of providing definitive insight into the optical scattering contributions of individual structures in the skin. In context of this discussion, this means that the contribution of Rayleigh scattering, caused by small-scale structures in the skin, to the overall scattering properties of a living skin specimen cannot be determined solely by examining the form of a reduced scattering coefficient computed for that specimen. Therefore, the observation that the reduced scattering coefficient of Verdel et al. \cite{18} does not have a Rayleigh scattering term does not necessarily imply that subsequent simulations employing this reduced scattering coefficient correspond to a living skin specimen whose internal structures do not exhibit Rayleigh scattering. It follows that the bluish vein appearance presented by Zoller and Kienle \cite{10} resulting from their simulations performed using the reduced scattering coefficient of Verdel et al. \cite{18} also does not necessarily provide insight into the contribution of Rayleigh scattering, caused by small-scale structures in the skin, to the appearance of a corresponding living skin specimen with a subcutaneous vein. Consequently, we do not believe that the results provided by Zoller and Kienle \cite{10} are sufficient to refute the possibility that Rayleigh scattering has a major influence on the bluish appearance of veins.

5 Concluding Remarks

In this paper, we described the differences between the approaches employed by Van Leeuwen and Baranoski \cite{3} and by Zoller and Kienle \cite{10} in their investigations of the optical mechanisms responsible for the bluish appearance of veins. We also clarified several aspects of these two works and discussed the conclusions presented in both publications regarding the contribution of Rayleigh scattering to the bluish appearance of veins. More specifically, in our discussion of the work by Van Leeuwen and Baranoski, \cite{3} we underscored the fact that they did not definitively state that Rayleigh scattering has a major influence on the bluish appearance of veins. However, their findings, which were obtained using first-principles models, do provide support for the hypothesis that it can have such an influence. Subsequently, in our discussion of the work by Zoller and Kienle, \cite{10} we systematically examined their investigation approach to show why the results presented in their publication are not sufficient to refute the proposal that Rayleigh scattering has a major influence on the bluish appearance of veins.

We believe that future advances in this area will greatly benefit from the availability of more detailed in situ measured datasets describing the individual heterogeneous structures (e.g., fibrils and organelles) contained within complex human tissues such as skin and blood. These datasets will be instrumental to further the current understanding about the actual impact of these structures on the optical properties and appearance of these tissues.

References