In vivo assessment of dermal fibers by LC-OCT: variations with age, ethnicity and after application of skin care cosmetic products

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Abstract

The objective of this study was to investigate the influence of aging, ethnicity, and the use of anti-aging skincare products on fiber dermis characteristics. To achieve noninvasive high-resolution 3D images of the cheekbone's skin in women, line field confocal optical coherence tomography (LC-OCT) was employed. An algorithm for highlighting tubular structures based on Frangi's algorithm was developed to generate an automated 3D segmentation of the dermal fibers. From this segmentation different metrics were calculated: number and mean length of fibers, number of nodes/reticulations, density and anisotropy score of the dermal fibers. The findings indicated that with age, there was a decrease in the mean length of fibers and an increase in the anisotropy score. Ethnic differences were observed. The fiber network was denser in Asians than in Caucasians. This was evidenced by the greater number of fibers and their more extensive nodes. Nevertheless, the mean length of fibers and anisotropy score were found to be comparable between the two groups. The application of the skincare products resulted in an increase in the number and mean length of fibers, number of nodes, and fiber

network density after one and three months of application, accompanied by a decrease in the anisotropy score.

Keywords:

Dermal fibers; automated segmentation; skin aging; anti-aging skin care products; Line field Confocal Optical Coherence Tomography.

Introduction.

Understanding the complex behavior of dermal fibers is crucial for advancing dermatological science and improving skincare products. Dermal fibers play a pivotal role in maintaining the structural integrity and elasticity of the skin, and their behavior is influenced by various factors including aging, ethnicity, and the use of anti-aging skincare products. This article aims to explore these influences through two studies; the first study investigated the impact of aging and ethnicity (Asian and Caucasian) on fiber properties. The second study investigated the influence of serum and cream use in conjunction with one another on fiber characteristics. Images of the women's cheeks were acquired using LC-OCT. Image processing techniques coupled to a new procedure of automated 3D segmentation of dermal fibers were applied to the input images to first extract the information of interest, a procedure of an automated 3D segmentation of the dermal fibers was developed, and then compute various metrics such as number and mean length of fibers, number of nodes/reticulations, anisotropy score, and density of the fiber network.

Materials and Methods.

Clinical studies.

Two clinical studies were conducted as part of this research. The first study aimed to assess skin aging and compare Caucasian to Asian women. Two distinct age groups of volunteers were recruited. The first group consisted of 17 Asians and 16 Caucasians, aged between 20 and 30

years old. The second group comprised 10 Asians and 17 Caucasians, aged between 50 and 60 years old. The second study was conducted to assess the efficacy of anti-aging products over a three-month period in 31 female Caucasian participants aged between 30 and 65 years old. Subjects were asked to apply a serum and a cream on the whole face twice a day in normal conditions of use. The first application was carried out at the investigational center under control of the technical staff. Participants did not apply any skin products to their face on the mornings of the LC-OCT measurement days. The measurements were taken at three timepoints: t_0 (before the commencement of the care routine), t_1 (after one month) and t_3 (after three months).

The first study was conducted during the autumnal season, while the second study was conducted during the spring, in order to eliminate any potential influence of solar radiation on the skin's properties.

The two clinical studies were conducted in accordance with the Declaration of Helsinki.

Image acquisition.

LC-OCT (DAMAE Medical, France, Paris) is a non-invasive imaging technique based on interferometry and low-coherence light principles. LC-OCT generates high-resolution cross-sectional images of biological tissues, horizontal images and 3D stacks. The device produces vertical and horizontal sectional images at a rate of eight frames per second, with a depth penetration of approximately 500 µm. LC-OCT has an axial resolution of 1.1 µm, a lateral resolution of 1.3 µm, and a field of view of 1.2 mm×0.5 mm (vertical) and 1.2 mm×0.5mm (horizontal). In conjunction with microscopic imaging, the device offers a color macroscopic imaging modality along with real-time localization of the imaged region, facilitating precise targeting of the area of interest. This macroscopic surface image encompasses a 2.5 mm diameter field of view with a resolution of approximately 5 µm. [1]

This device was employed in this study to achieve high-resolution 3D images of the cheekbone’s skin. The procedure for in vivo human skin acquisition involves the application of a drop of paraffin oil between the skin and the glass window of the handheld probe. [1]

The resulting 3D stacks were processed using an in-house developed algorithm for dermal fiber segmentation and various metrics were calculated. All imaging acquisitions were conducted at controlled room laboratory temperature, with the volunteers lying down on a medical chair to minimize motion artefacts during acquisition.

Image processing.

Multiple image processing techniques were applied to the images in order to first extract the dermis fibers, and then compute different metrics. LC-OCT images (Figure 1A1) were grayscale images with dimensions 1200x1200x300 μm^3 for the first study and 1276x500x300 μm^3 for the second study. The images acquired in the two studies exhibit disparate dimensions due to the evolution of the system between the two studies. The dermis volume analyzed in the two clinical studies had a thickness of 40 µm and commenced at the dermal-epidermal junction (Figure 1B). The first step of the process (Figure 1C) involved the enhancement of pixels corresponding to dermal fibers via a methodology based on generic multiscale Hessian-based measures [2], which represents a generalization of Frangi's vesselness measure. This approach is a well-known technique in the medical field for enhancing blood vessels visualization in an image. The approach also enabled to extract the direction of the enhanced structures at each pixel. The second step involved the segmentation of fibers on the enhanced image. This was achieved by applying an automated threshold that separated the pixels into foreground (fibers) and background classes. To be less sensitive to the threshold value which has influence on the thickness of the fibers network, the third step involved the extraction of the centerline of fibers (Figure 1D) by using a binary thinning algorithm [3], which reduced each shape to a one-pixel-wide line. The final step involved the computation of metrics based on the centerline of fibers. In order to compute the numbers of nodes, mean length and number of fibers, it was first necessary to identify the points in the centerline that corresponded to nodes in the fiber network. This was accomplished by iterating through all the points in the centerline and identifying those having more than two neighbors (Figure 2B). The total of these points, gave the number of nodes. The

fibers were then counted and their mean length was measured. Furthermore, two distinct fiber classes were identified. Long fibers were defined as having at least one node, while short fibers lack nodes. Fiber network density was calculated by multiplying the number of fibers by their mean length, and dividing by the total number of pixels in the dermis volume. The anisotropy score, which is a measure of the irregularity of fiber orientation in the dermis, was calculated by analyzing the direction matrix (Figure 2A) (generated in the first step). When the score is equal to zero, the fibers have a pluridirectional organization, and when is equal to 1, the fibers have a unidirectional organization.

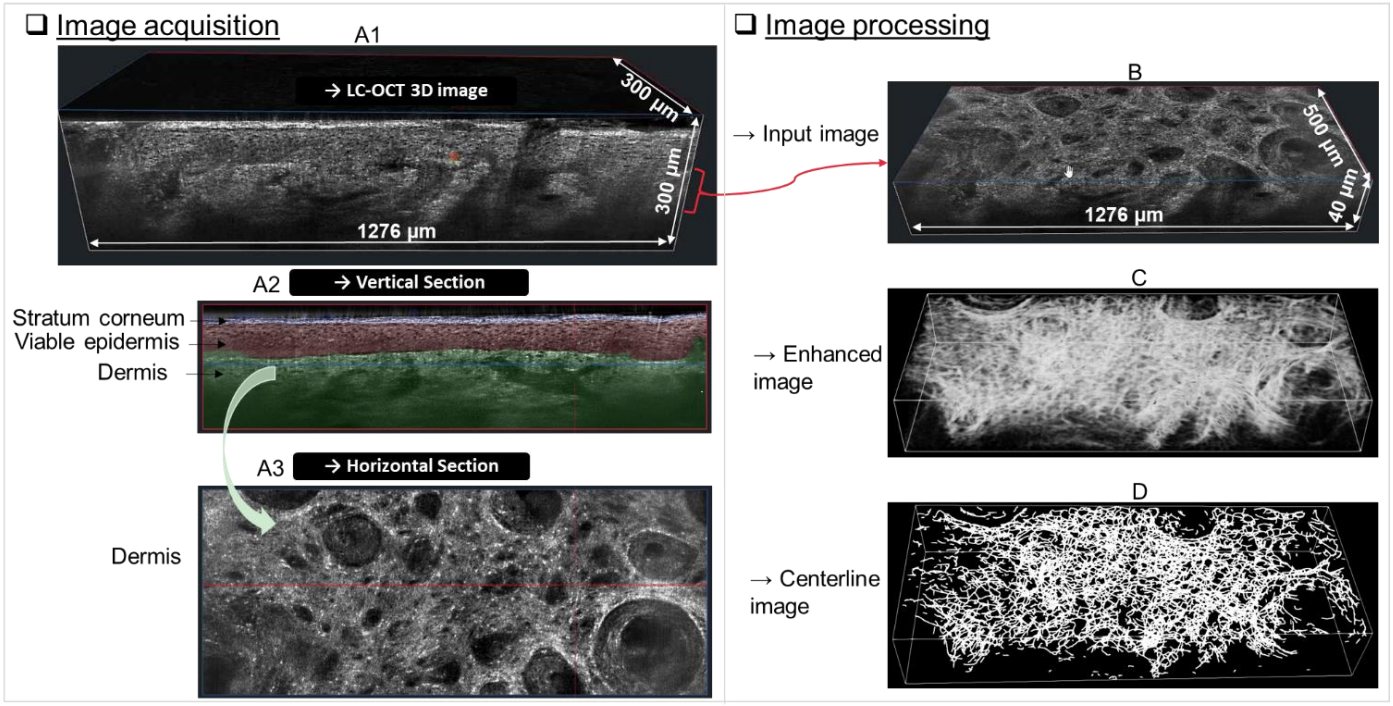

Figure 1: Characteristics of LC-OCT images (A1, A2 and A3) and the main steps of image processing (B, C and D).

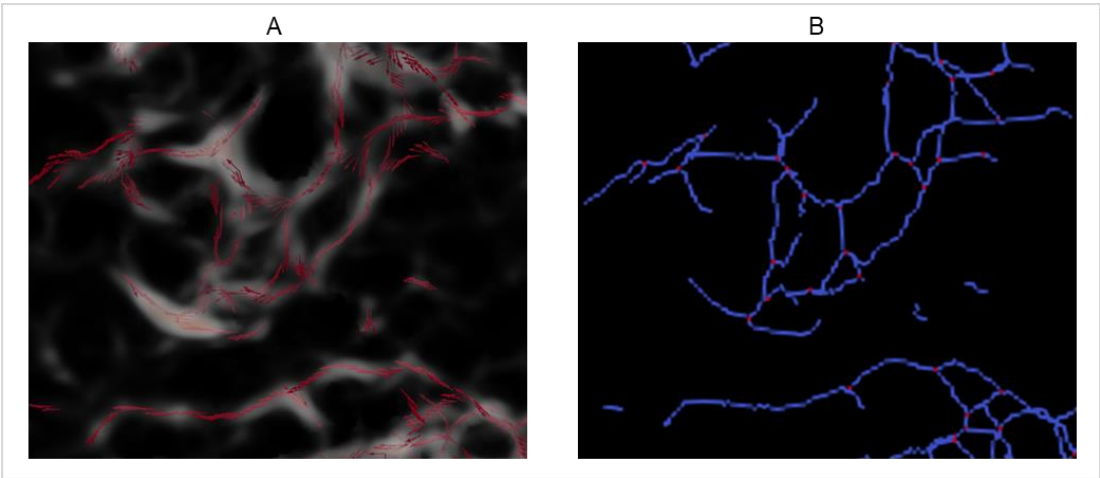

Figure 2: Computed directions on the enhanced image (A) and centerline nodes points (B)

Statistical analysis.

Statistical analyses have been run on R (version 4.2.2).

To determine whether there was a significant effect of the age factor (young vs aged) and/or the ethnicity factor (Caucasian vs Asian) on dermal fiber characteristics (numbers, mean length, nodes, density and anisotropy score), a two-ways ANOVA was performed or, a Scheirer-Ray-Hare test (a non-parametric alternative to the two-factor ANOVA (Scheirer-Ray-Hare test) according to the distribution of the parameters studied .

To determine whether there was a significant change in the characteristics of the dermal fibers after skin care products application, mean comparison tests for paired data have been carried out between the different studied timepoints. Student, Welch or Wilcoson tests were applied according to the distribution of the parameters.

Graphical visualizations (boxplots) have been produced to illustrate the analysis results.

Results.

In the first study, ie. the difference of dermal fibers according to the two age groups and ethnicity, the results obtained on the cheekbone’s skin were the following ones:

The number of fibers was not statistically different between both studied aged groups, while their mean length decreased significantly with age. This observation holds true for both long and short fibers, as well as when analyzed together (Figure 3). The number of the nodes and the density were not significantly different, while the anisotropy score increased statistically with age (Figure 4).

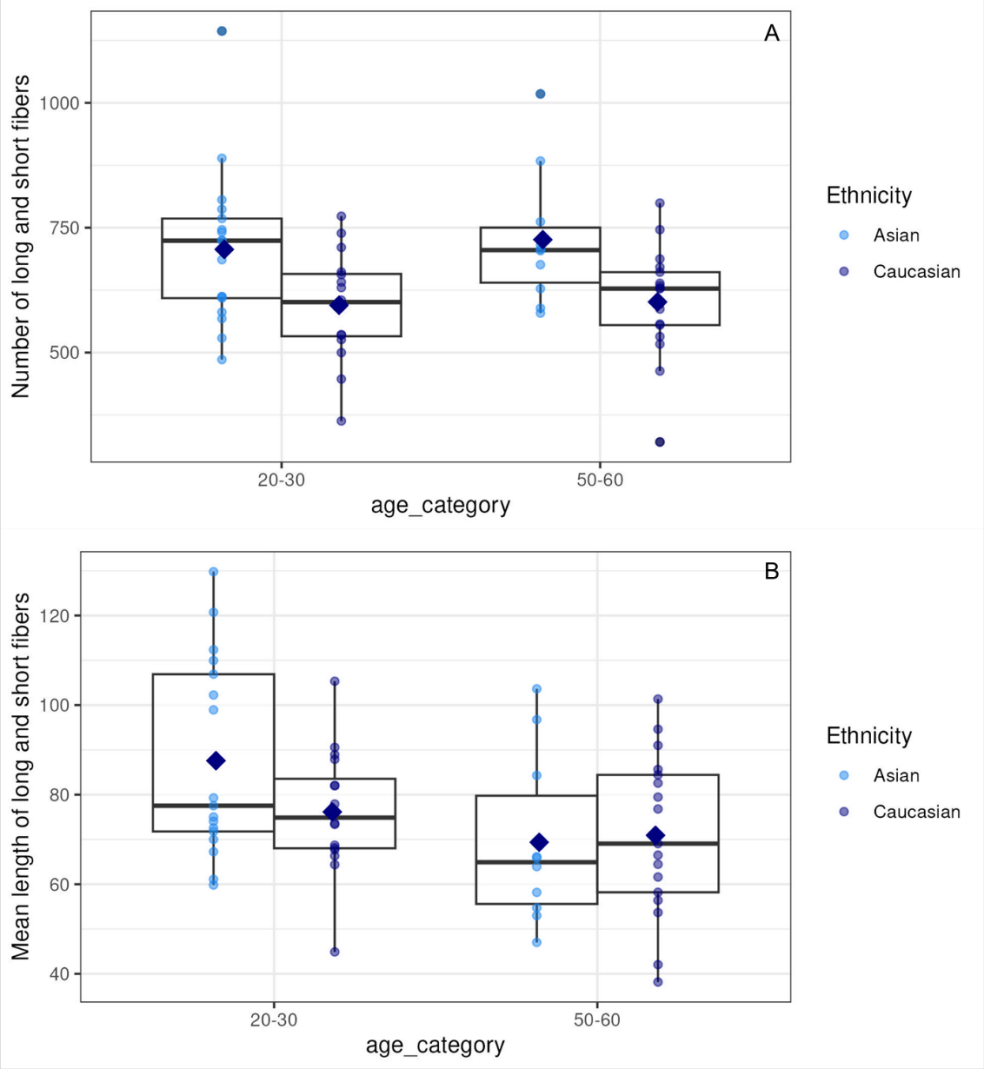

Figure 3: Number (A) and mean length (B) of dermal fibers according to age and ethnicity.

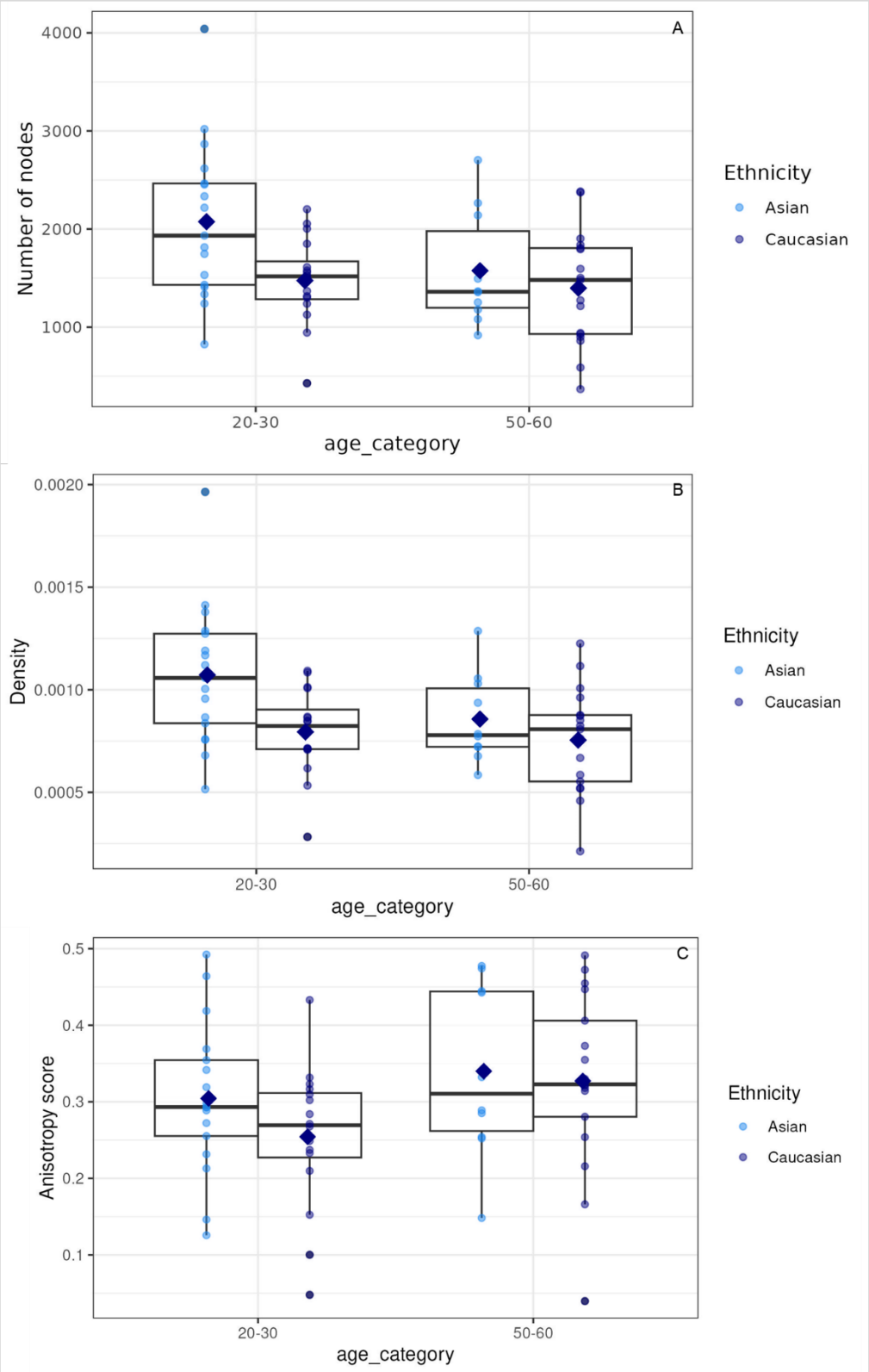

Figure 4: Number of nodes (A), density (B) and anisotropy score (C) for fiber network according to age and ethnicity.

The comparison between the two ethnic groups revealed that the number of fibers was significantly higher in the Asian group compared to the Caucasian group, while their mean length were not significantly different. This observation was consistent for both long and short fibers, as well as when analyzed together (Figure 3). The number of nodes and density were significantly higher in the Asian group than in the Caucasian group (Figure 4A and 4B). The anisotropy score was not statistically different between the Asian and Caucasian groups (Figure 4C).

In the second study, ie. the effect of an anti-aging skincare products, the results showed that after one month of application, the number of long fibers was significantly decreased, while the number of short fibers was not significantly different. The total number of the two types of fibers decreased but not significantly (Figure 5A). The mean length of the long fibers exhibited a significant increase, while the mean length of the short fibers exhibited a significant decrease. Consequently, the mean length of total fibers exhibited a significant increase (Figure 5B), despite a decrease in the mean length of short fibers. This was due to the fact that the mean length of long fibers was considerably greater than that of short fibers. Consequently, the results were influenced by the variation of long fibers when they were analyzed together. The number of nodes exhibited a notable increase, and the density exhibited a tendency to rise. In contrast, the anisotropy score exhibited a significant reduction (Figure 6). These results were consistent with the second point of measurement when comparing the baseline (t_0) and three months later (t_3). The sole distinction between the two periods was that the density exhibited a marked increase after three months, whereas it exhibited a tendency to increase after one month. No further changes were observed after three months compared to one month (Figure 3 and 4).

The statistical analysis results for both clinical trials are summarized in Table 1.

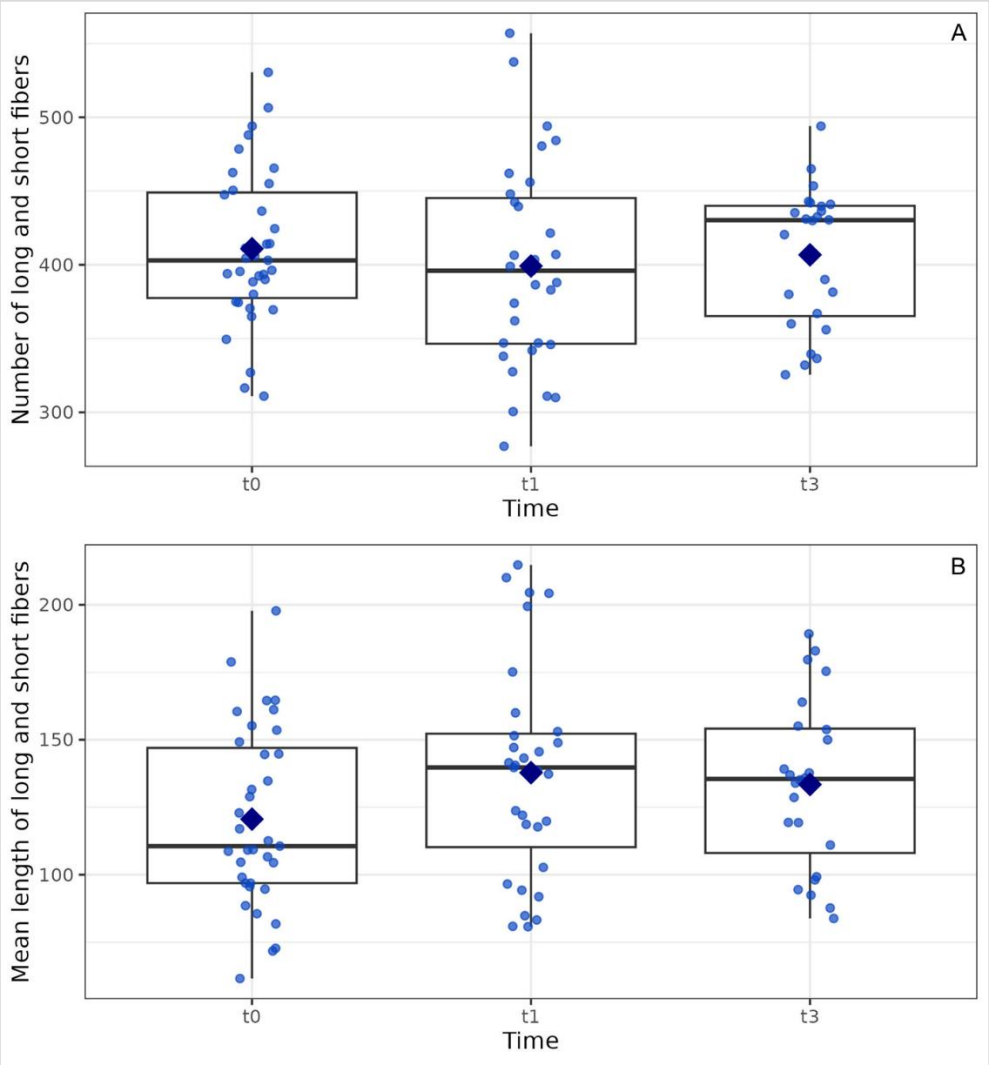

Figure 5: Number (A) and mean length (B) of fibers after one and three months of use of skin care products.

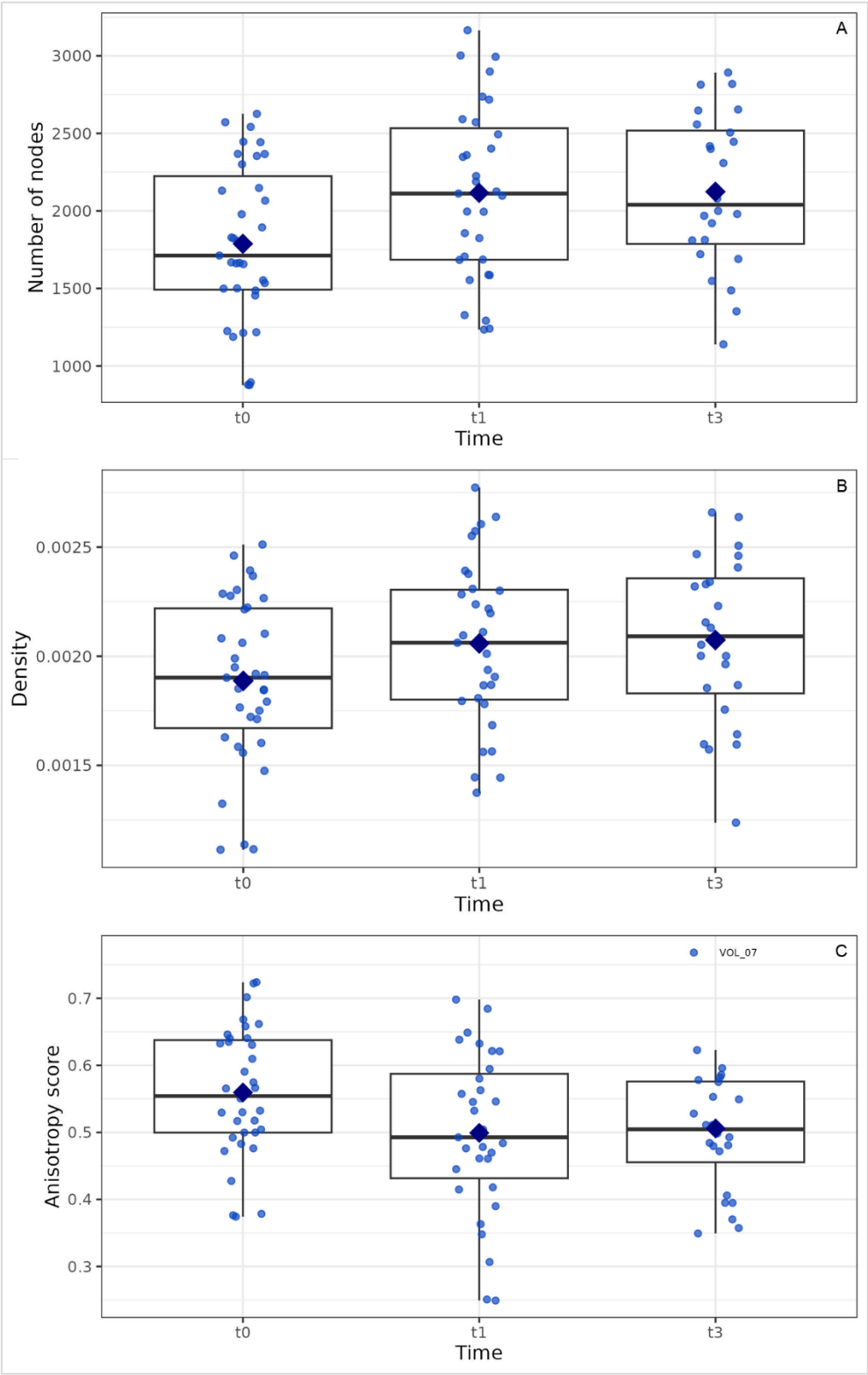

Figure 6: Number of nodes (A), density (B) and anisotropy score (C) after one and three months of use of skin care products.

Table I: Statistical analysis results. S: Significative. NS: No significative. T: Trend. P: p-value. Y: Young. O: old. A: Asian. C: Caucasian.

	Clinical study n°1: Skin aging and comparison between two ethnic groups		**Clinical study n°2: Effect of an anti-aging product on fiber dermis**	
Metrics	**Age effect**	**Ethnicity effect**	**t_0 vs t_1**	**t_0 vs t_3**
Number of long and short fibers	Y= 652±167 O= 648±130 P= 0.7167 (NS)	A= 714±147 C= 598±109 P= 0.0009 (**S**)	t_0= 412±50 t_1= 399±68 P= 0.294 (NS)	t_3= 407±46 P= 0.306 (NS)
Number of long fibers	Y=151±40 O=147±34 P= 0.9522 (NS)	A= 165±39 C= 137±26 P= 0.0014 (**S**)	t_0= 101±18 t_1= 89±23 P= 0.012 (**S**)	t_3= 90±16 P= 0.005 (**S**)
Number of short fibers	Y= 501±129 O= 500±100 P= 0.6530 (NS)	A= 549±112 C= 462±86 P= 0.0011 (**S**)	t_0= 311±36 t_1= 310±48 P= 0.878 (NS)	t_3= 317±34 P= 0.944 (NS)
Size of long and short fibers	Y= 82±20 O= 70±18 P= 0.0310 (**S**)	A= 81±22 C= 73±16 P= 0.2395 (NS)	t_0= 121±31 t_1= 138±39 P= 0.045 (**S**)	t_3= 133±31 P= 0.015 (**S**)
Size of long fibers	Y= 310±111 O= 262±81 P= 0.0818 (T)	A= 308±119 C= 273±67 P= 0.2395 (NS)	t_0= 462±149 t_1= 596±224 P= 0.006 (**S**)	t_3= 580±182 P= 0.001 (**S**)
Size of short fibers	Y= 15±0.6 O= 14±0.7 P= 0.070 (T)	A= 15±0.8 C= 14±0.6 P= 0.5359 (NS)	t_0= 14±0.7 t_1= 13±0.6 P= 0 (**S**)	t_3= 13±0.7 P= 0.004 (**S**)
Number of nodes	Y= 1785±803 O= 1463±570 P= 0.1176 (NS)	A= 1890±791 C= 1435±522 P= 0.0139 (**S**)	t_0= 1804±486 t_1= 2116±546 P= 0.009 (**S**)	t_3= 2124±487 P= 0 (**S**)
Density	Y= 0.09%±0.03 O= 0.08%±0.02 P= 0.1082 (NS)	A= 0.1%±0.03 C= 0.08%±0.02 P= 0.0060 (**S**)	t_0= 0.076%±0.01 t_1= 0.082%±0.01 P= 0.072 (T)	t_3= 0.083%±0.02 P= 0.007 (**S**)
Anisotropy score	Y= 0.28 ±0.1 O= 0.33±0.1 P= 0.0438 (**S**)	A= 0.32±0.1 C= 0.29±0.1 P= 0.2218 (NS)	t_0= 0.55±0.1 t_1= 0.50±0.1 P= 0.004 (**S**)	t_3= 0.51±0.1 P= 0.003 (**S**)

Discussion.

The findings from the two studies provide significant insights into the behavior of dermal fibers concerning aging, ethnicity, and the effects of anti-aging skincare products.

Our study demonstrated that aging impacted significantly the mean length of fibers. The reduction in fiber length with age could mean that the fibers undergo fragmentation/degradation.

Indeed, Fligiel et al. [4] employed biochemical and ultrastructural approaches to demonstrate that photodamaged skin exhibited extensive collagen fragmentation and clumping, while sun-protected aged skin exhibited similar damage, but with less extensive damage in individuals aged 80 years or older. In contrast, sun-protected young skin exhibited minimal damage. The number of fibers and of nodes, and the density of the network fibers were not significantly different, indicating maybe that the structural framework of fibers was still preserved. It was anticipated that these parameters would decline with age; however, the findings of this study do not confirm this hypothesis. The most probable explanation for this discrepancy is that the age range of older individuals in this study was between 50 and 60 years old, which is not sufficiently advanced to demonstrate a significant decline. We can understand that the aging process begins with fiber fragmentation, then later with advanced fragmentation, the network fibers would have a loss of connectivity and a decrease in density. Future research should focus on understanding the behavior of skin aging after 60 years old. The observed increase in the anisotropy score indicated a change in the directional organization of the fibers. As they age, dermal fibers tend to orient themselves in a preferred direction. In their study, Nguyen et al. [5] employed polarized-FTIR imaging to examine the morphological alterations in dermal collagen, during chronological aging. They found that type I collagen fibers undergo reorientation, becoming parallel to the skin surface with age.

The comparison between Asian and Caucasian groups revealed notable differences in collagen fiber characteristics. Asians exhibited a higher number of dermal fibers and connectivity and greater density compared to Caucasians. The observed outcomes can be attributed to two primary factors, as previously documented in the literature. The first point to be considered is the amount of melanin present in different ethnic groups. Individuals of Asian and Black ethnicities possess greater quantities of melanin than those of Caucasian ethnicity. This may confer a more protective effect on the Asian dermis. The second point is that Black and Asian individuals have a thicker and more compact dermis than white skin. [6], [7] Despite these differences in number

and density, the mean length of the fibers and the anisotropy score did not differ significantly between the groups.

The second study, evaluating the effect of anti-aging skin care products, showed promising results in modifying the structural properties of dermal fibers. After one month of application, the number of long fibers decreased and their mean length and number of nodes increased. This could indicate the formation of new connections between these fibers, perhaps in the form of bridges. The number of short fibers was not significantly different, yet their mean length decreases. With the increased density of the fiber network, it is plausible that the short fibers were associated with the long ones, while at the same time, new fibers, shorter than the originals, were synthesized. This would explain why the number of short fibers remained constant while their mean length decreased. Indeed, the active ingredient utilized in the formulation of anti-aging skincare products has retinol-like activity. The literature mentioned that Retinoids enhance the collagen production in the papillary dermis [8] and increase the thickness of dermis [9]. The reduction in the anisotropy score indicated a reorganization of the fiber network due to the establishment of new connections. This implied that fibers tend to orient themselves in various directions in comparison to their initial state. Humbert PG et al. [10] showed that after topical application of 5% vitamin C cream there was a significant increase in skin microrelief density, i.e. a decrease in the anisotropy index, and a reduction in deep furrows. The skin relief parameters were determined by the authors using silicone rubber. It is noteworthy that these improvements were sustained for a period of three months, which indicates the long-lasting efficacy of the products.

Conclusion.

In this work, we have developed a new automatic algorithm for dermal fiber segmentation and introduced relevant metrics for a deeper understanding of the dermal matrix state. The combination of LC-OCT and dermal fiber characterization provides a powerful tool to gain a

deeper understanding the impact of age and ethnicity on dermal fibers, and how anti-aging skincare products influence the structural properties of dermal fibers.

The results demonstrated that skin aging is associated with fiber fragmentation and a more anisotropic fiber network. Asians have a denser fiber network than Caucasians, with fibers more numerous and more reticulated. The two cosmetic products applied daily to the face induced an increase in fiber synthesis and the creation of new connections between fibers and a more isotropic fiber network.

This study introduces a new approach that paves the way for various research on the dermis, including the study of a wide range of age groups, different body sites, gender differences, all ethnicities, the influence of factors such as sun exposure and air pollution, as well as in vivo evaluation of cosmetic product efficacy.

Acknowledgments.

We gratefully acknowledge the DAMAE group for providing access to the LC-OCT measurement device. Their support and the use of this advanced equipment were essential to the success of our research.

Conflict of Interest Statement.

NONE.

References.

1. Jdid R, Pedrazzani M, Lejeune F, et al (2024) Skin dark spot mapping and valuation of brightening product efficacy using Line-field Confocal Optical Coherence Tomography (LC-OCT). Skin Res Technol 30 13612–13623.
2. Antiga L (2007). Generalizing vesselness with respect to dimensionality and shape. The Insight Journal. http://hdl.handle.net/1926/576.

3. Homann H (2007). Implementation of a 3D thinning algorithm. The Insight Journal.. http://hdl.handle.net/1926/1292.
4. Fligiel SE, Varani J, Datta SC, et al (2023) Collagen Degradation in ged/Photodamaged Skin In vivo and After Exposure to Matrix Metalloproteinase-1 In Vitro. J Invest Dermatol 120:842–848.
5. Nguyen TT, Eklouh-Molinier C, Sebiskveradze D, et al (2014) Changes of skin collagen orientation associated with chronological aging as probed by polarized-FTIR micro-imaging. Analyst 139:2482–2488.
6. Ling L.C (2010) Aging in Asian Skin. In: Farage, M.A., Miller, K.W., Maibach, H.I. (eds) Textbook of Aging Skin. Springer, Berlin, Heidelberg 1019–1024.
7. Vashi NA, de Castro Maymone MB, Kundu RV (2016). Aging Differences in Ethnic Skin 9 :31–38.
8. Kohl E, Steinbauer J, Landthaler M, et al (2011). Skin ageing. J Eur Acad Dermatol Venereol 25: 873–884.
9. Taihao Q (2023). Human Skin Aging and the Anti-Aging Properties of Retinol. Biomolecules 13: 1611–1614.
10. Humbert PG, Haftek M, Creidi P, et all (2003). Topical ascorbic acid on photoaged skin. Clinical, topographical and ultrastructural evaluation: double-blind study vs. placebo. Exp Dermatol 12:233–237.