Contribution of human skin topography to the characterization of dynamic skin tension during senescence: morpho-mechanical approach

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Abstract. The structuring of the dermis with a network of collagen and elastic fibres gives a three-dimensional structure to the skin network with directions perpendicular and parallel to the skin surface. This three-dimensional morphology prints on the surface of the stratum corneum a three dimensional network of lines which express the mechanical tension of the skin at rest. To evaluate the changes of skin morphology, we used a three-dimensional confocal microscopy and characterization of skin imaging of volar forearm microrelief. We have accurately characterize the role of skin line network during chronological aging with the identification of depth scales on the network of lines ($z \leq 60\mu m$) and the network of lines covering Langer's lines ($z > 60$ microns). During aging has been highlighted lower rows for elastic fibres, the decrease weakened the tension and results in enlargement of the plates of the microrelief, which gives us a geometric pertinent indicator to quantify the loss of skin tension and assess the stage of aging. The study of 120 Caucasian women shows that ageing in the volar forearm zone results in changes in the morphology of the line network organisation. The decrease in secondary lines ($z \leq 60$ µm) is counterbalanced by an increase in the depth of the primary lines ($z > 60$ µm) and an accentuation of the anisotropy index.

1. Introduction

The longevity of life is a recent unsurpassed demographic phenomenon in the history of the humanity and impact of which will be considerable. The analysis of ageing of populations and its social consequences requests an interdisciplinary approach. By gathering capacities of research in these different domains, it becomes possible to treat in a transverse way the big questions linked to ageing and to longevity, by understanding better all the factors that drive to the fragility of the old tissues (origin of diseases, of dysfunctions and handicaps).

Old age is inevitable and a natural period of life characterized by a decrease in physical function, loss of social role as an adult, changes in physical appearance and routing to a gradual decrease in capacity. Aging is part of a continuing evolution in the course of human development, rigorously following embryogenesis, puberty and maturation. Throughout this process, the organs develop into effect after a specific time. The "program" of aging is probably mediated by the endocrine hormones, neurotransmitters acting on certain target organs. Finally the cell is genetically programmed and the programming could be compared to a turtle horloge. A turtle is programmed for 100 years, a monkey for 20 to 25 years and 120 years for humans. For against this life expectancy is compromised by damage to genetic or acquired by a malfunctioning biological or enzymatic cell. The first signs that we perceive aging are changes in the body that they depend on internal changes. It is important to note that the rate of aging varies with individuals. Thus the change of hair color, which gradually become gray and then white, and the appearance of wrinkles are a reflection of the inevitable passing of time. Skin problems, which are more common in the elderly than in younger individuals, are not only related to the fact that the skin is exposed to a long remained more or less cumulative dose of ultraviolet rays, but also to change in structures the skin itself (connective tissue, collagen, elastic fibres, etc..).

The skin is a set of grouped cells in the form of a flexible and resistant fabric, composed of several layers, and covering the whole body. The skin consists of three distinct parts: the stratum corneum, the epidermis (epi = above) whose main role is to protect the body, and the dermis, only one with vessels allowing nutrients (nutrients carried by the blood) to diffuse to the epidermis. In an adult, the skin weighs between 3.5 kilograms and 4.5 kilograms. Its total area up to two square meters. It is highly vascularized and also has a large number of glands producing sweat (sweat glands), sebaceous glands (sebum secreting = fatty substance that protects the skin) and nerve receptors for tactile sensations and pressure. This cover (integument) very flexible but strong helps protect the body against external aggression (infections, temperature variations, etc. ...). The thickness of the skin varies between 1.5 and 4 mm depending on the region of the body in question. The epidermis covering the soles and palms is thicker than the rest of the body. Below the dermis lies the hypodermis, also called superficial fascia. It consists of adipose tissue (fat) tissue and looser than the dermis. The dermis has a role to adapt to the movements of structures located below it (muscles, tendons, and fascia) but also protect the body shots, thanks to its constitution fat. It is at this level that is fat stores of the body, which accumulate in humans in the abdomen, and women on the thighs and breasts. 1) The epidermis contains different varieties of cells, including keratinocytes (which are the most numerous), and melanocytes.

1.1. Skin Lines Network
The skin surface shows a specific topography depending on the anatomical site, age and sex. In general, the skin morphology presents a 3D network of lines, who by his organization expresses all the multidirectional tensions of elastic fibres and the collagen beams. Micro-lines, primary lines, fine wrinkle and wrinkle represent, in fact, the special organization of collagen bundles and elastic fibres in the superficial dermis, and there is a relationship between the morphology of skin lines and elastic network. Different functions can be attributed to the lines network. The first function is the retention and drainage canals of the sebum and sweat. They collect preferentially and retain for long time the substances applied to the skin: they are thus preferential sites of percutaneous absorption. This reservoir function, allow the applied topical products to be stored on the skin surface and then eventually to diffuse in its different layers. The second function is mechanic, during ageing the depth, width, density and orientation of skin lines change. Some lines become more marked; they evolve progressively in marked anisotropy connected to the decrease of the elasticity of the collagen fibres.

1.2. Skin Tension and Anisotropy of Skin Lines Network
When analysing the mechanics of skin in vivo, a significant property is its natural tension. Discovered by Dupuytren [1], and mapped by Langer [2], the non-uniform skin tension lines exist. Langer has identified these lines by puncturing the skin with a circular device, figure (1). The wounds then assume an elliptical shape and by joining the major axes of the ellipses a system of lines can be drawn, Some authors propose other methods to obtain these lines, such as wrinkling of the skin by Borges[3].

Skin resistance to traction predominates in the Langer's lines direction and varies with body site. On all body sites, the skin tension is greater in the direction of Langer's lines, figure (2).
This phenomenon is the source of the Young's modulus anisotropy[4], whose distribution angle shows a maximum in the Langer's lines axis [5],. This result favour a similar orientation of the elastic fibres involved in the skin. Assuming that the fibres are independent, it has been calculated that, on the calf, 76% were in the direction of Langer lines and 5.1% perpendicular [6]. Of course the distribution concerns only the elastic fibres, which are parallel to the skin surface. Observation of the dermis with scanning electron microscopy confirms this data [7]. In retracted skin the collagen bundles look tortuous, with no special direction, and sinuous elastic fibres are fixed to them in several places, especially in their concave portion.

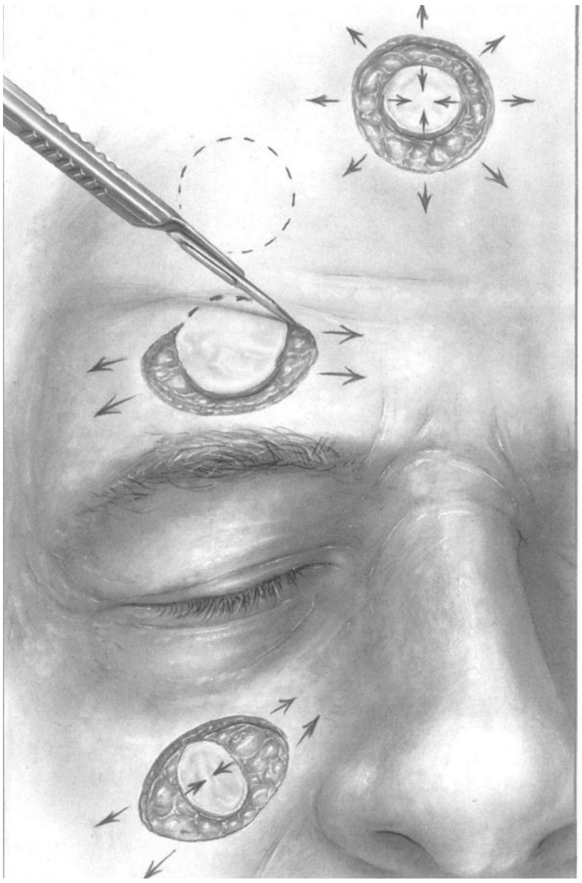

Figure 1. Detection of Langer's lines: evolution of circular incisions on the face by Waldorf [4].

In non-retracted skin, the thinnest collagen bundles as well as the elastic fibers are straightened in the direction of the Langer's lines and almost parallel; the thickest bundles remain tortuous and oriented in all directions, but their shape seems to be modified by the traction from the oriented bundles and fibres. Contrary to common belief in the past, Langer's lines do not reflect anisotropy of the collagen density but anisotropy of the reticular dermis collagen bundles' direction and elastic fibres' tension.

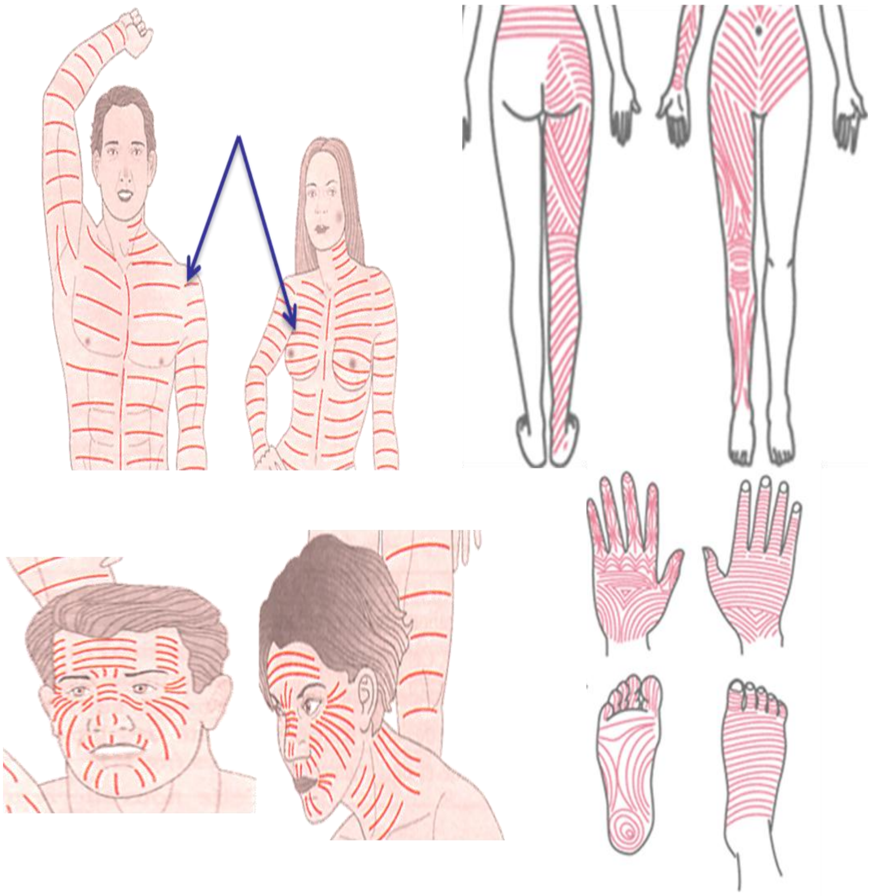

Figure 2. Schematic representation of Langer's lines on different areas of the body.

1.3. Identification of Langer's Lines

The morphology of lines network reflects the intrinsic tension of the skin, which is distinct from the additional tension induced by increases in volume of the underlying tissues, such as muscle contraction, edema, or particular posture which stretches the skin. The identification of Langer's lines must therefore, be made on relaxed skin.

Several methods of identification of Langer's lines were developed, as evidenced by the following works of literature:

- Stark's Method [8]

According to the definition, the direction of the maximum tension can be found by stretching the skin in several directions with an equal force, the direction of minimum elongation is that of Langer's lines. Stark developed a simple device comparable to a compass: two branches with claws at their extremities part spontaneously by 30 mm under the action of a 14.2g/mm spring. He could measure the elongation of the skin into eight directions quickly, each measurement requiring only 1.5 seconds.

- Borges' Method[3]

This similar method is less accurate, but even quicker, and consists of creasing the skin between the thumb and the index in all directions until the furrows are regular and parallel. They follow Langer's lines. In the other directions, they are impeded by the skin tension that makes them irregular.

- Barbenel's Method [9]

The measurement of the extensibility of the skin using the suction method is valid only if slip page of the skin into the suction chamber is presented. If, inversely, this movement is facilitated, the most extensible direction of the skin will appear easily, that is to say perpendicularly to Langer's lines. If the contour of the chamber is drawn in during suction, when the chamber is removed, an oval outline is observed instead of a circle and its main axis corresponds to Langer's lines.

- Skin micro- topography : Zahouani's method [10,11,12]

Apart from the palms and soles, the skin mirorelief is made of plateaus separated by valleys. The latter are roughly parallel and oriented in different directions, and this layout is characteristic of each body area. The direction of the deepest valleys matches Langer's lines. There may be one or two other preferential directions, indicating an ordered no orthogonal mechanical anisotropy. This method has an advantage over the others as it is insensitive to extrinsic skin tensions. Its physiological interpretation is simple. The cutis is normally retracted (skin tension) and extensible, whereas the epidermis has none of these properties. Therefore, the epidermal creasing responsible for the micro relief appears to be a transformation of tension allowing the creases to be flattened by stretching them. The superficial dermis, an intermediary zone between epidermis and cutis, is precisely the place where the skin relief begins. This mechanical transduction is one of its functions.
It is this approach that we developed to study the chronological aging of human skin. The method uses the wealth of three-dimensional imaging of the skin and the possibility of linking a signature printed on the surface of the stratum corneum with the organization in volume of the different skin layers.

2. Morphological evolution of skin lines network of caucasian french women during aging

Several studies have demonstrated changes in the network of lines with age, leading to deepening of certain lines and the disappearance of others [13-22]. However, quantitative and detailed descriptions of the modifications of skin lines with age are rarely reported in the literature since most of the published results are based on standard parameters which give an overall description of the topography of any surface, without specificity for the skin morphology. For this reason, we have adapted a 3D confocal microscope working with a high vertical and lateral resolution, which enables precise characterisation of the skin lines network. Negative skin replicas were taken with silicone rubber (Silfo®, Flexico Ltd, England) from 120 Caucasian French women equally divided into six age groups (20-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years and 70-80 years). Replicas were taken from the women's left volar forearm at the same pre-determined area, after a 30-minute rest period in an environmentally controlled room (temperature: 21 ± 2ºC and relative humidity: 50 ± 5%).
Resolution refers to the smallest distance the confocal microscope can accurately measure. It can be considered in terms of lateral or and vertical resolution. The vertical resolution value of the confocal microscope is about 0.01 µm with a vertical range of 1000 µm. .The lateral resolution depends on the quality of displacement in the plane x, y. In the case of skin aging, the lateral resolution which was used is 1 µm in the directions x, y.
Figure (3) shows the evolution of skin volar forearm morphology of Caucasian women aged 20 to 80 years.

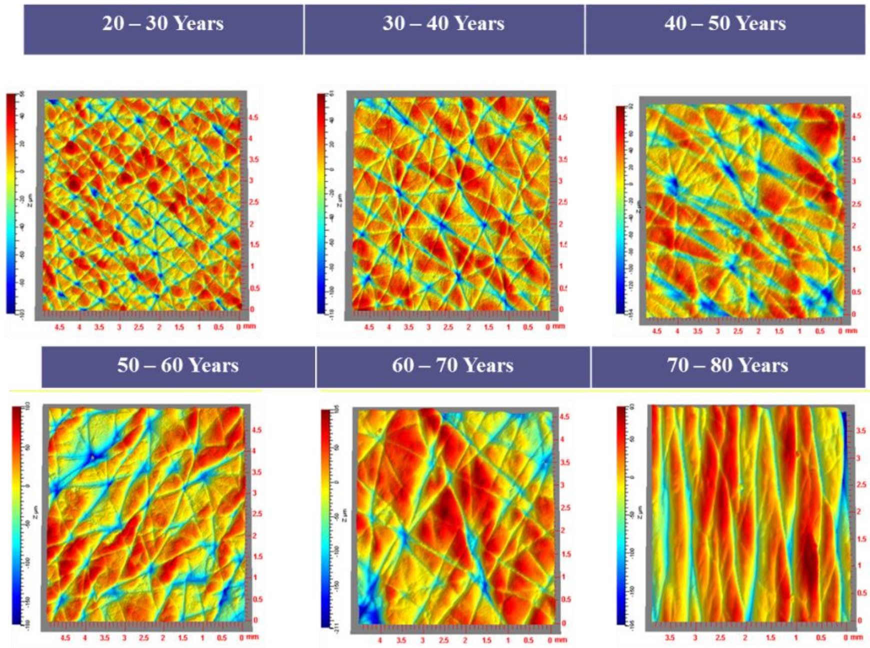

Figure 3. Morphology of skin relief of Caucasian women during aging (volar forearm site).

The images reflect the three-dimensional character of skin lines network. The scale height is expressed here by a colour scale. The deepest lines are expressed by the Colour Blue, heights that are at the top of plates expressed through into colour red – black. The full scale (pick to valley) can reach 500 µm

2.1. Multi-Scale Analysis of Skin Lines Network Morphology

Microscopic observations have shown that skin morphology contains a network of lines whose organisation reflects the multidirectional tensions of elastic and collagen fibres in the superficial dermis (11). Hashimoto (12) gave a precise four-level classification of the line network scales: (I) The primary lines are clearly marked and are between 20 and 100 µm deep, (II) The secondary lines are more discrete and correspond to a depth of 5 - 40 µm, and are perpendicular to the primary lines, (III) The tertiary lines correspond to the corneocyte border (about 0.5 µm), (IV) The quaternary lines correspond to the morphology of each corneocyte (about 0.05 µm). Tertiary and quaternary lines cannot be seen without magnification.

To study the transformation of the 3D skin line network during aging, it is necessary to identify all its local motifs. A motif of skin line is defined by the association of two peaks separated by the hollow of a valley, the height is determined by the difference between the highest peak and the hollow of the valley.[23-24], figure (4) :

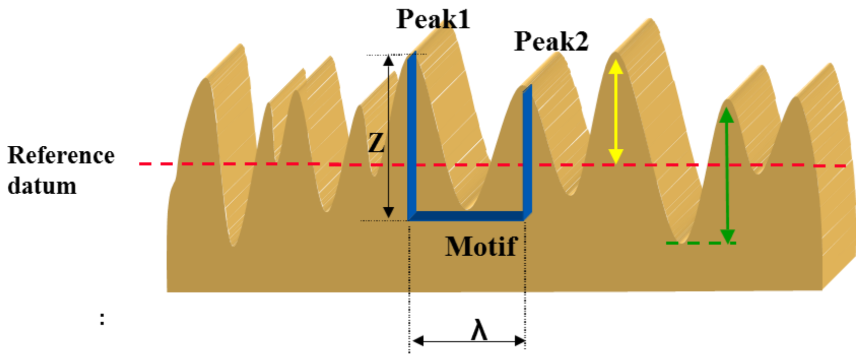

Figure 4. Definition of tension lines patterns.

The width of the motif (λ) is given by the distance between both peaks. The direction of the motif which coincides with the main direction of the line is defined in the orthogonal direction at the maximal variation of the local gradient, figure (3).

This quantitative approach enables us to follow the evolution of the different families of lines in relation to age. The depth Z depends on the skin site and two classes of depth $Z1 \leq 60$ µm and $Z2 > 60$ µm were adopted for the volar forearm aging. This choice was fixed after sampling every 10 µm as class depth. The results showed a marked decrease in density of the family lines of depth less than 60 µm and a net increase in density of the family of lines at depths exceeding 60 µm:

- $Z1 \leq 60$ µm, related the tension effect of elastic fibers network
- $Z2 > 60$ µm, related to Langer's line

Analysis of the morphology of skin tension lines of Caucasian women aged between 20 and 80 years, shows significantly decrease of the density of lines of depth <60 microns and an augmentation of deep lines beyond 60µm. This important result presented in figure (5) shows the mechanical role of elastic fibres in maintaining skin tension and firmness of young skin. This elastic relaxation mechanism of the reduction of elastic fibers according to age is the basis of the phenomenon of the appearance of wrinkles.

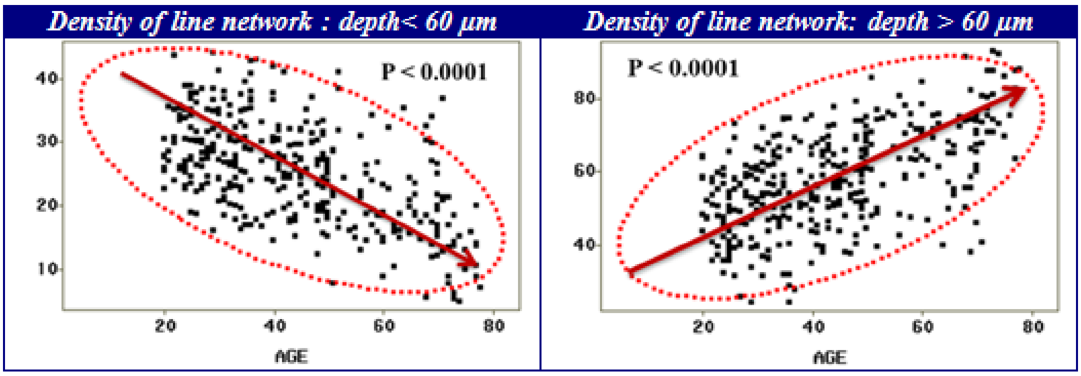

Figure 5. Evolution of the scale lines of tension during aging.

2.2. Changing the Orientation of Lines and Anisotropy during Aging

For better identification of the shift of skin lines network, we privileged to work on the rest state of the skin of the forearm by characterizing the image of the skin relief at different stages of aging. The methodology is to identify the local orientation of a pattern of lines and quantify the overall direction of the network of lines over the 0 ° direction taken as the axis of the body from head to foot [25, 26, 27]. Two conditions are adopted: i) the direction must coincide with the local normal of the plan formed by the three features of the motif, figure (6), ii) if the first peak is point A, the hollow point B and the second peak point C, the normal vector N is approximately collinear to the vector operation:

$$\vec{N} = \vec{AB} \wedge \vec{BC} \tag{1}$$

$$\vec{N} = \vec{CB} \wedge \vec{CA}$$

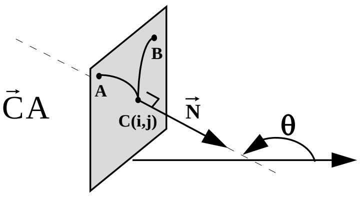

Figure 6. Detection of the skin line direction.

The direction of the motif must coincide with the direction of the valley which is composed of a succession of motifs, it is necessary to know if the hollow C(i,j) belongs to the move of the neighbouring points, figure (7). To achieve this, the intersection points between half a straight line stemming from C(i,j) and the nearest points are noted. If there is an even number of these intersection points, then the point is outside the direction of the skin line, otherwise the hollow is in the direction of the skin line. The number of iterations of this procedure is equal to the number of valley points of the detected motifs, and the three elements of the motif Z, λ and θ are memorised for each motif; these three parameters will be the fundamental components to build the 3D morphological tree of skin lines network.

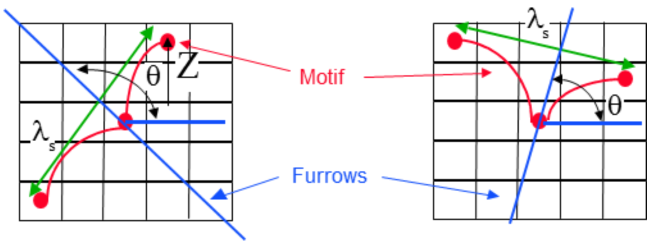

Figure 7. Orientation of local motif and skin line tension.

This approach quantifies the morphology of skin lines according to their orientation and their depth. The orientation distribution of the skin lines was quantified as a compass rose. This graphic representation plots in 20° intervals the density of lines between 0° and 180°, with the body axis used as the principal axis of orientation, figure (8). Thus, the density of lines in a triangular part corresponds to the percentage of line patterns which have this orientation.

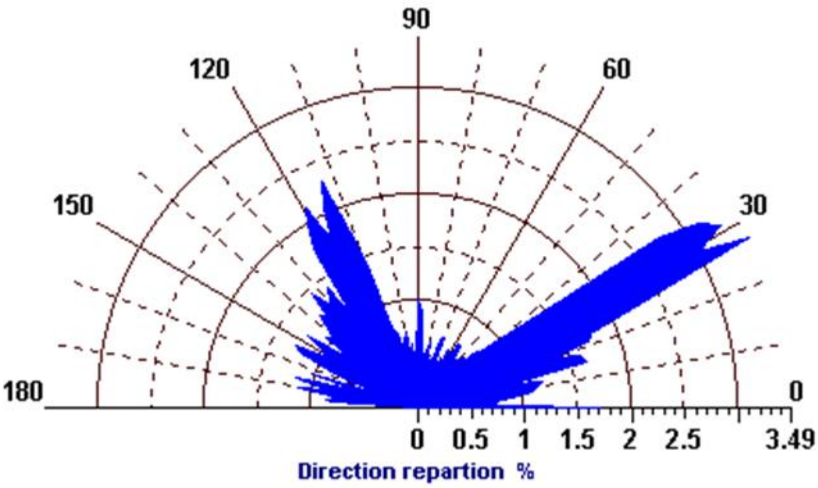

Figure 8. Anisotropy of kin lines network of the forearm of a subject 40 years.

2.3. Dynamic Rotation of Skin Line Network during Aging

To monitor the rotational dynamics of skin lines during aging, the overall results for the densities of line orientation from 20 to 80 years were collected for a comprehensive representation of the dynamic change of direction in function of age. We have chosen to represent this change for those aged under 60 and over 60 years. Figure (9) shows the rotational dynamics and the significant decrease in tension lines in the directions between 90 and 180 ° and the establishment of a marked anisotropy between the directions 20 and 60 degrees. This result demonstrates the relationship between the voltage loss of elastin fibres and lower voltage lines printed on the plates of the relief and the depth is less than 60 microns.

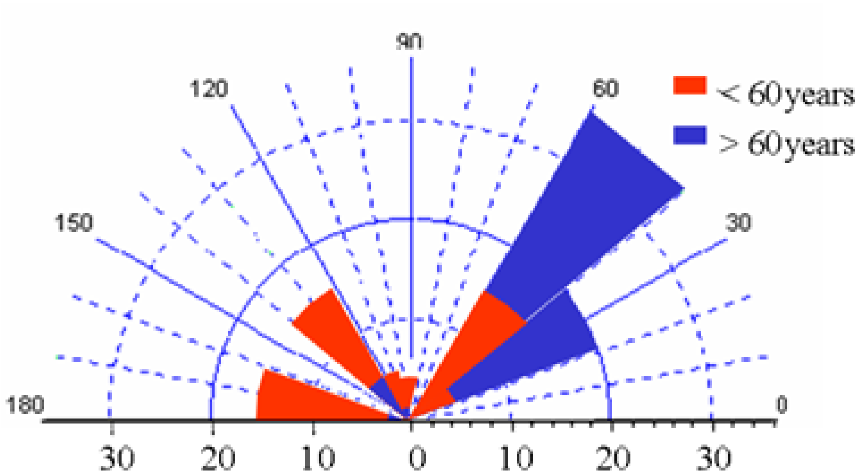

Figure 9. Aging effect on the dynamic rotation of skin line network of 120 Caucasian women.

2.4. Anisotropy Index of Skin Lines Network during Aging

The parameter resulting from the information on the density of lines according to their depth and orientation is the anisotropy. To assess the degree of the skin lines anisotropy during ageing, we introduced an anisotropy index from direction roses. A completely anisotropic surface gives a rose oriented into only one angular sector. Conversely, a perfectly isotropic surface leads to a circular direction rose. In consequence, if N is the number of angular sectors between 0 and π, the anisotropy index (A.I) can be defined as [26,27]

$$AI = \frac{1}{2}\frac{\sum_{i=0}^{N-1}|R_i - S/N|}{S - S/N}*100 \quad (2)$$

where R_i is the rose value corresponding to angular sector i and [26]

$$S = \sum_{i=0}^{N-1} R_i \quad (3)$$

S/N should be the R_i value for all i in the case of a perfectly isotropic surface and the factor 1/2 derives from the fact that an R_i value greater than S/N must be exactly compensated by lower values. As a result of the increase of the density of lines deeper than 60 µm and the diminution of the density of lines < 60 µm, results in the increase of the anisotropy index significantly during aging, figure (10).

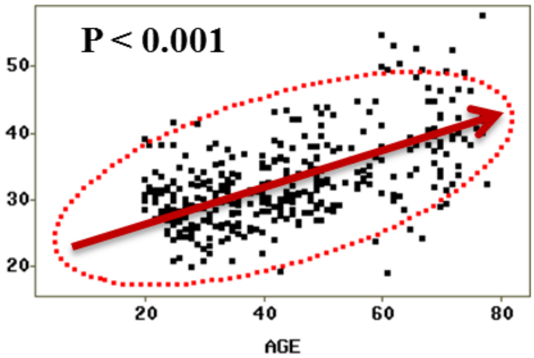

Figure 10. Aging effect on the skin line anisotropy index of 120 Caucasian women.

2.5. 3D Reconstruction of Skin Line Network: Volumetric Anisotropy and Tree of Skin Tension Network [27, 28, 29, 30]

The dermis is the layer of living skin. It is a supporting connective tissue rich in fiber which gives the skin elasticity and strength. The dermis contains the appendices of the skin. Histologically, the dermis can be divided into two layers: the papillary and reticular layer. Dermis and epidermis are closely meshed into each other through many outgrowths of the surface ripples of the dermis called papillae. The papillary dermis contains many nerve endings (thermo-receptors, tactile receptors). The reticular dermis consists of a network of collagen bundles (thick, wavy, perpendicular to the basal membrane) more visible because more dense within the reticular dermis. The network of elastic fibres which underlies the undulations of collagen fibres bundles and around the latter is anchored to their concavity. The reticular dermis is the strongest part of the dermis. His mobility results from unfolding the undulating collagen fibres bundles of allowing their extension and their return to their original position by the action of elastic fibres.
This structuring of the dermis with a network of collagen and elastic fibers gives a three-dimensional structure to the skin network with directions perpendicular and parallel to the skin surface. This three-dimensional morphology prints on the surface of the stratum corneum a three dimensional network of lines which express the mechanical tension of the skin at rest.

The approach developed specifically to the skin morphology allows the identification of the lines network anisotropy at different scales of depth and orientation. For each plane at a certain depth of the skin surface, are determined three parameters of the point belonging to the line of tension: the density of depth z, the width of the line and the rose of directions between 0 and 180° [27, 28, 29]. The figure (11A) represents the identification of the network of skin tension lines in different directions about a 25 year old. The depth of the skin lines is illustrated by the range of colours from blue to red. One can distinguish the family of skin lines printed on the plates: secondary lines (colours of green, yellow, and red correspond to a variation of depths between -17 microns and 50 microns). The family of skin tension lines in the main colour blue are in a scale between -17 and -84 microns. The identification of the orientation of skin lines in different directions is illustrated by the figures: 11B, 11C, 11D, 11E. The figure (12), illustrates the anisotropy distribution versus the depth of skin network families.

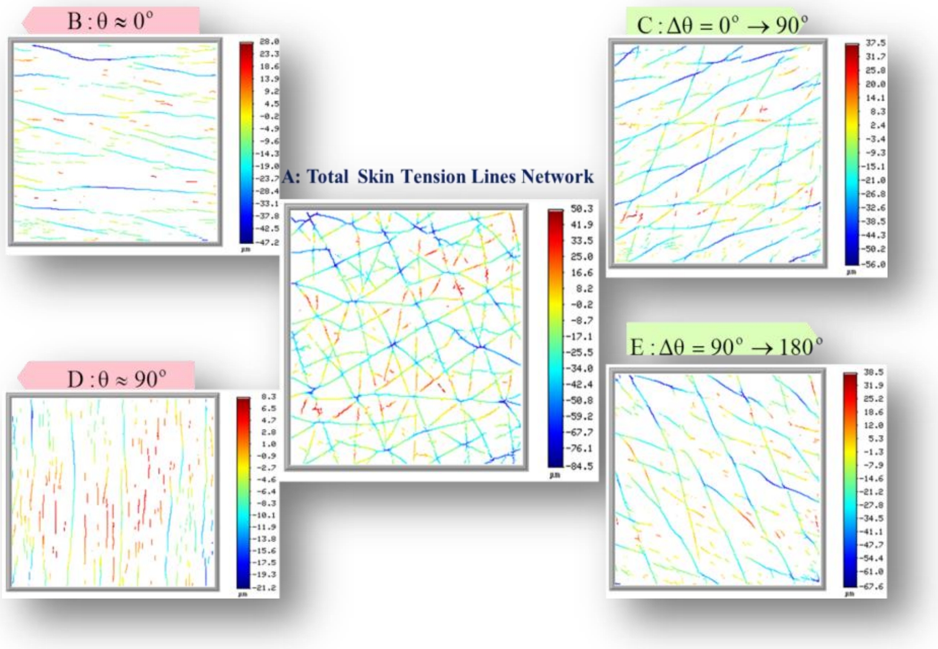

Figure 11. Network of skin tension lines.

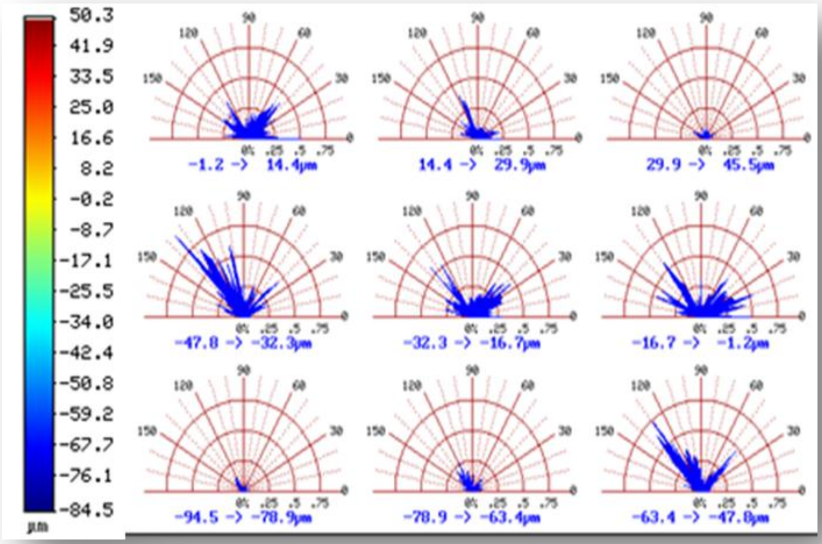

Figure.12. Reconstruction volumetric skin tension lines anisotropy.

2.6. Morphological Tree of Skin Line Network [28, 29, 30, 11]

Appropriate and quantitative representation of skin line network has been developed. It allows reconstructing all network lines as a morphological tree. Each trunk of tree represents the density of lines in a given direction and for a given depth, figure (13)

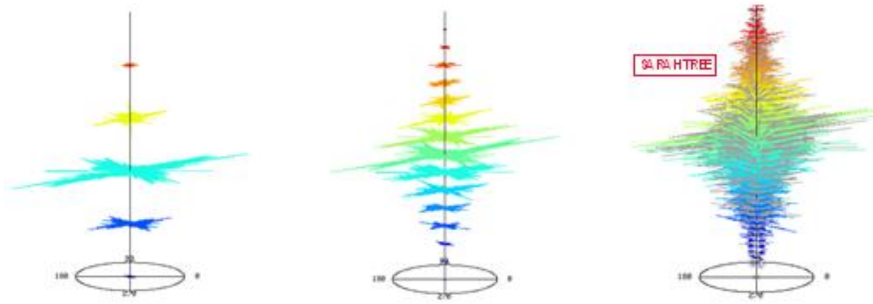

Figure 13. 3D Reconstruction: Tree of skin tension lines network.

One family of lines is described as a branch of the tree, in relation to its depth and direction. With this original method, it is possible for the first time to quantify the different scales of skin line accurately and to follow the morphological changes of the surface in relation to age. This multi-morphological decomposition of line network can be used to assess mechanical tension of elastic fibres and collagen bundle during ageing. In the other hand, this approach can be used as a preventive test for certain diseases of the elasticity of the skin. The examples of figure.(14) shows the use of this approach in the analysis of the transformation of the 3D line network during ageing [11,26].

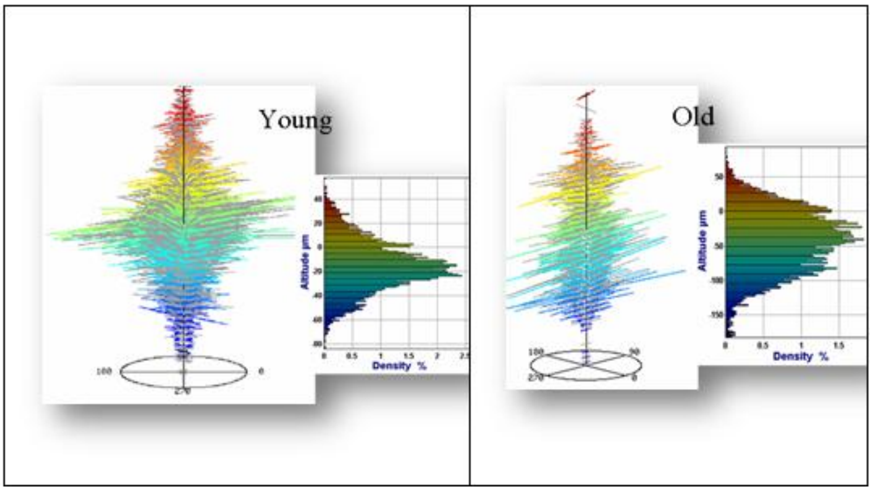

Figure 14. Aging effect on the Tree of skin tension lines network [11, 26, 27, 28].

3. Conclusion

Changes of dermal collagen and elastin content are characteristic for skin aging as well as for pathological skin conditions. The formation of lines and wrinkles in light-exposed areas throughout the body, such as the face, throat and hands is a well-known sign of skin aging. Lines and wrinkles are influenced by both intrinsic and extrinsic factors. The numerous intrinsic factors are age, gender, genetic disposition and race. In exposed and unprotected zones of the body such as on the skin of the hands and the face, extrinsic factors such as UV light, weather and climatic influences, nutrition, tobacco and alcohol abuse, effect the formation of lines and wrinkles.

With increasing age, the physiology and appearance of the human skin will change. Alterations in structure, loss in tightness, smoothness and a decrease in the skin's functional capacity are phenomena which may be attributed to the aging mechanism. An increase in dryness and thus roughness as well as a loss in elasticity and even pigmentation are also a sign of increasing skin aging. Wrinkles on flaccid skin develop with growing age. There is a decline in the subcutaneous fatty tissue. Today, relatively little is known about the exact biomechanical processes of skin aging.

Primarily, changes in the skin's appearance are a result of a general aging process of the connective tissue of the subcutis. This leads to an atrophy of the epidermis which adjoins the papillary layer and to an irregular decrease in the elasticity of the elastic nets which are structures accompanying the collagen fibres in the connective tissue. As a result of the changed amount and chemical composition of the basic substance of the connective tissue, a loss of liquids is the result, which consequently leads to a decrease in glycosaminoglycans, the basic structures of the connective and supporting tissues. As a consequence, the youthful turgor, i.e. the skin's tension, is lost. Melanocytes disintegrate or lose close contact to epidermal cells and finally lead to a spotted pigmentation of the skin.

To evaluate these changes, we used the characterization of skin imaging of microrelief. The study of 120 Caucasian women shows that ageing in the volar forearm zone results in changes in the morphology of the line network organisation. The decrease in secondary lines ($z < 60$ µm) is counterbalanced by an increase in the depth of the primary lines ($z > 60$ µm) and an accentuation of the anisotropy index. This phenomenon is known to be accelerated by actinic radiation (extrinsic photoageing) which increases the degradation of the elastic fibres.

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