Characterisation of knee ageing by fringe projection, standardised pictures and viscoelastic methods

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Abstract.

Skin ageing and its quantification is very well known on face. However, other sites are also very visible, and the knee is one of them. With age, knees become drier, wrinkled and suffer of ptosis. Our aim was to set up illustrative and quantitative methods to show and measure knee wrinkle changes through ageing and to get a reproducible and reliable method for, then, the evaluation of anti-ageing products.

We developed specific methods, first is a standardised acquisition with a photographic bench, second consisted in performing 3D acquisitions by fringe projection equipment (AEVA). Moreover, we used existing devices for testing skin visco-elasticity and density (ElastiMeter®, Cutometer® and Ultrasound DermaScan® C). 30 female volunteers aged 21 to 71 years old were recruited and submitted to various acquisitions on knees.

The photographic and the fringe projection benches allowed us to build up a 2D and 3D atlas with six age categories. For both roughness and elasticity analyses, we saw a high correlation with age. On the contrary, our try-out with ultrasound device did not show any clear differences in the dermis skin density between young and aged volunteers. We confirmed that wrinkles and elasticity on the knee are age dependent. Moreover, amongst various quantification methods, we found that fringe projection and Cutometer® parameters are the best to precisely quantify knee ageing.

Keywords: knees; wrinkles; ageing; evaluation.

Introduction.

There is only a few researches on ageing of the knee but it has been demonstrated that on this region, wrinkles increase and elasticity decreases with age [1]. Another study showed a correlation between observed laxity severity and age [2].

Laxity of the skin is caused by sagging of subcutaneous structures and, like wrinkles, is principally due to the loss of dermal extracellular matrix constituents, such as collagens and elastin. Moreover, ageing promotes a low moisturisation of knees which are already drier than other part of the body due to a lowest sebum secretion [3]. Weight changes, high BMI and sun exposure are also positively correlated with skin knee degradation [2]. Obviously, movements and frictions undergone by knees are negative factors that cumulate with age.
Wrinkled and flaccid skin of knees are now becoming an important cosmetic concern for many people. Surgical options have been available for many years but recently non-invasive treatments aiming to tighten the skin have been developed.
The principle of many treatments is to improve dermal strength and elasticity by remodelling the dermis with new collagen and elastin. This can be done by using targeted energy to penetrate dermis and form precise thermal coagulation points. Some devices are available with energy from a variety of sources including monopolar and bipolar radiofrequency [4], broadband and laser light sources, ultrasound and micro-focused ultrasound [5, 6]. Another treatment consists of an injection of diluted calcium hydroxyapatite to stimulate the synthesis of collagens and elastin and to increase neovascularisation [7]. In addition, several products exist and are safely used by the cosmetic industry to improve dermis and epidermis properties, such as plant extracts or small and well characterised peptides. Later are well known to trigger collagen and elastin synthesis or to reinforce epidermal barrier functions [8-11].
For treatment efficacy evaluation, or people classification, specific scales were done, for laxity [2], for laxity and cellulitis [12] or for wrinkles [1]. To our knowledge, only one study was focused on knee evaluation with metrological devices like fringe projection or cutometer® [1].
Our objective was to set up illustrative and quantitative methods allowing to show knee wrinkle changes through ageing or, conversely, after treatments. For this, 2 methods based on 2D / 3D images were developed and 3 existing devices for skin visco-elasticity and density were tested on a panel of-volunteers of different ages.

Materials and Methods.

Panel

All subjects were informed about the objective of the study and provided a written informed consent. The study respected the spirit of Good Clinical Practice (GCP). 30 female volunteers aged 21-71 y/o (mean 51 y/o) were recruited, and 3 age groups were constituted (7 “young” < 40 y/o, 13 “middle” 41 to 60 y/o, and 10 “old” >60 y/o). To avoid any weight influence, volunteers were selected with a Body Mass Index between 18 and 24 and had no history of recent weight changes. Because of possible artifacts, only volunteers without arthrosis or scars on knees were selected. Moreover, volunteers were asked not to apply any cosmetic the day of evaluation.

Study design

The study was conducted under controlled temperature (20-24°C) and humidity (30-70 %). For all the participants, measures and acquisitions were done on the upper and middle part of right and left knees.

Photographic system

A photographic bench HeadScan V04 with a body module (Orion TechnoLab™) was used to set up the pictures system. It is composed of a camera D7200 (Nikon) and 2 flashes ELC Pro HD500 (Elinchrom), the shooting system is remotely controlled by a computer.

The position of the body, and especially the pelvis and feet, is very important to have optimal and standardised pictures. In order to control both part of the body we developed a system to wedge the hips of the volunteers and thus avoid any movement. Moreover, a measuring device was set up to specifically position the feet angles. Reproducibility of pictures over time was evaluated by comparing pictures taken at 30 minutes intervals.

Fringe projection system

A fringe projection system AEVA-HE (Eotech) associated with a Visio 4D bench (Eotech) were used to set up the 3D acquisition of knees. The sensor is equipped with 2 lenses to perform an acquisition with following parameters: 200x150x100 mm field of view, 80 µm lateral resolution and 4 µm vertical resolution.

As for the photographic bench, we have managed the position of pelvis and feet by adding the same measuring device to position the feet angles, and a new walker system to eliminate any movement during the acquisition. Reproducibility of measurements over time was evaluated and has given good results.

3D images were taken, and a multiple analysis was done to obtain classical roughness parameters but also features density based on local curvature detection (Fig. 1). Analysis was adapted to upper- or middle-part specificities.

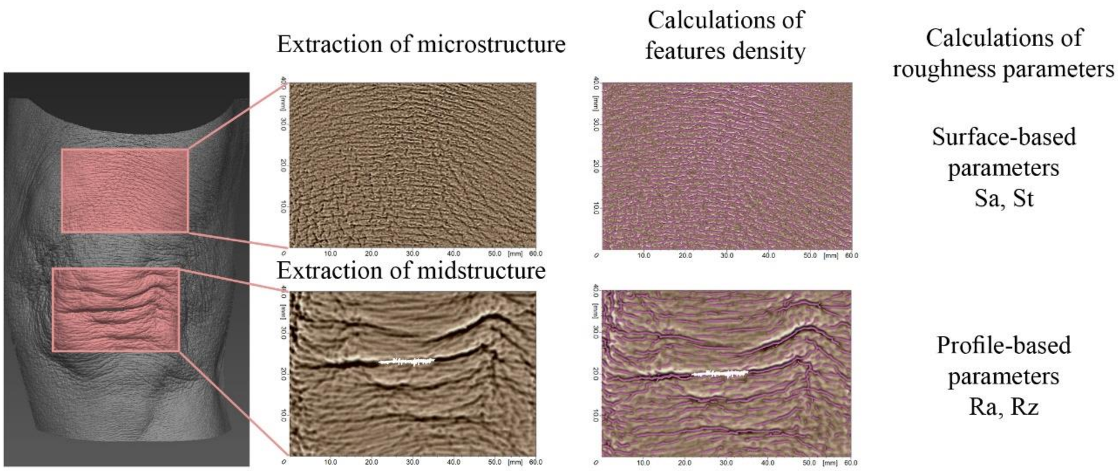


Figure 1: Skin topography analysis pathway

Skin elasticity

Skin elasticity was measured using Cutometer® MPA580 (Courage + Khazaka) and ElastiMeter (Delfin technologies), two well-known and validated devices [13]. For both devices, 3 measures for each site were done.

For measures with the Cutometer®, a 6 mm probe was used and the device was set up to generate negative pressure of 450 mbar during 1s (on-time) following by no pressure during 1s (off time). This cycle was repeated ten times. All the parameters were studied (R, Q and F parameters). For ElastiMeter, a 0.6 mm indentometer was used and the Instant Skin Elasticity (ISE) was calculated.

Skin density

A 20 or 50 MHz ultrasound – DermaScan® C (Cortex) was used to evaluate dermis thickness and echogenicity. Successive pictures were taken in one single “cineloop” acquisition and the Image J software (National Institute of Health) was used for extraction of representative pictures to study or quantify thickness or density of the dermis.

Statistical analysis

Excel 365 was used to perform statistical analysis. A two-tailed unpaired *t*-test was used to compare age groups and a Pearson's correlation coefficient (R^2) was used to see whether parameters were statistically correlated with age.

<u>Results.</u>

Photographic system

For the method reproducibility , grading by 2 trained experts demonstrated a good (41 %) or very good (45 %) repositioning of volunteers.

The pictures were split in 6 ages categories (20 – 30 – 40 – 50 – 60 – 70 y/o) and the most representative picture for each age category was chosen to build an atlas of knees (Fig. 2)

Figure 2: 2D pictures of knees obtained with photographic bench and representing each age category

This result shows that changes appear clearly around 40 y/o and keep going with age.

The changes on upper part of the knee preferentially involve skin roughness, texture and fine wrinkles. On the middle part of knee, changes involve large and deep wrinkles but also sagging.

Fringe projection system and roughness analysis

An identical atlas with same volunteers was built with 3D acquisitions (Fig. 3)

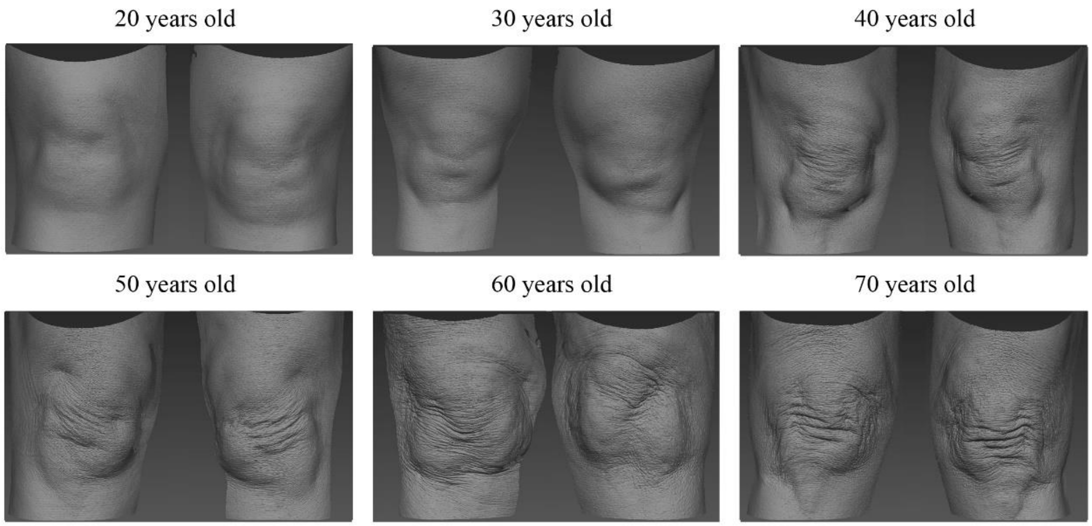
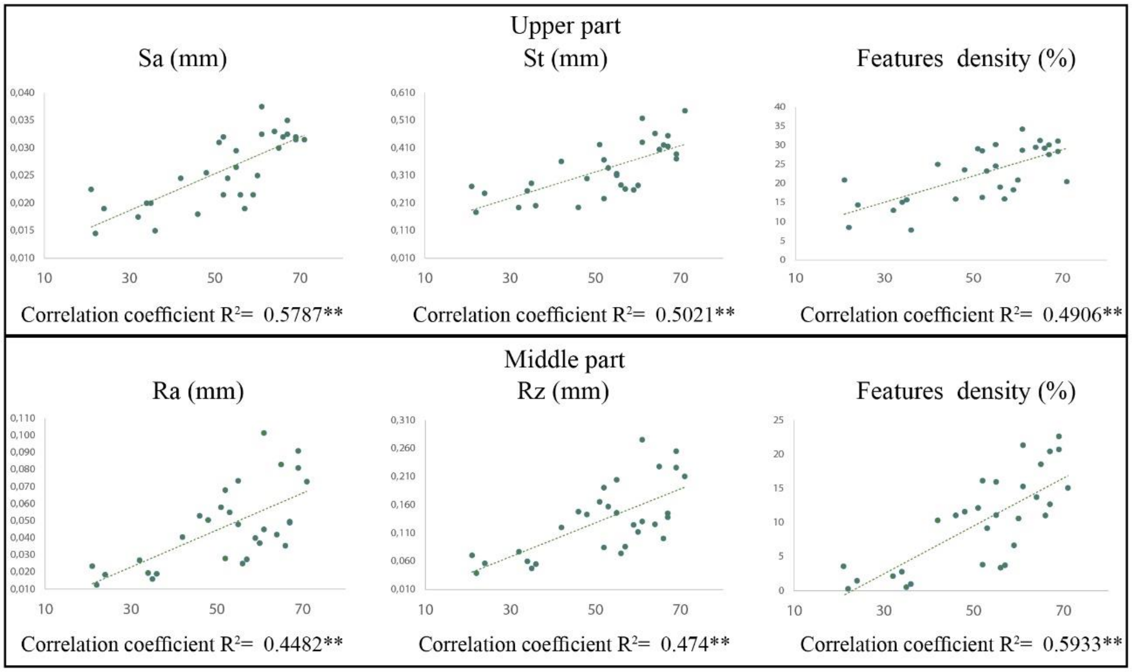


Figure 3: 3D images of knees obtained with fringe projection and representing each age category

For the upper part of knee, roughness Sa and St, as well as feature density showed a significant correlation with age with a correlation coefficient R^2 egal to 0.5787, 0.5021, 0.4906 respectively (Table 1). Identically, for the middle part of knee, Ra, Rz, and Features density showed a significant correlation with age with R^2 egal to 0.4482, 0.474, 0.5933 respectively (Table 1).

Table 1: Correlations between topographic parameters and age, **$p<0.01$



The difference between “young”, “middle” and “old” was significant for all the parameters (Fig. 4). In all cases, roughness or feature density increased in middle age category compared to young category and in old category compared to middle category. It can be noticed that in middle part, the increase between young and middle category was higher compared to upper part.

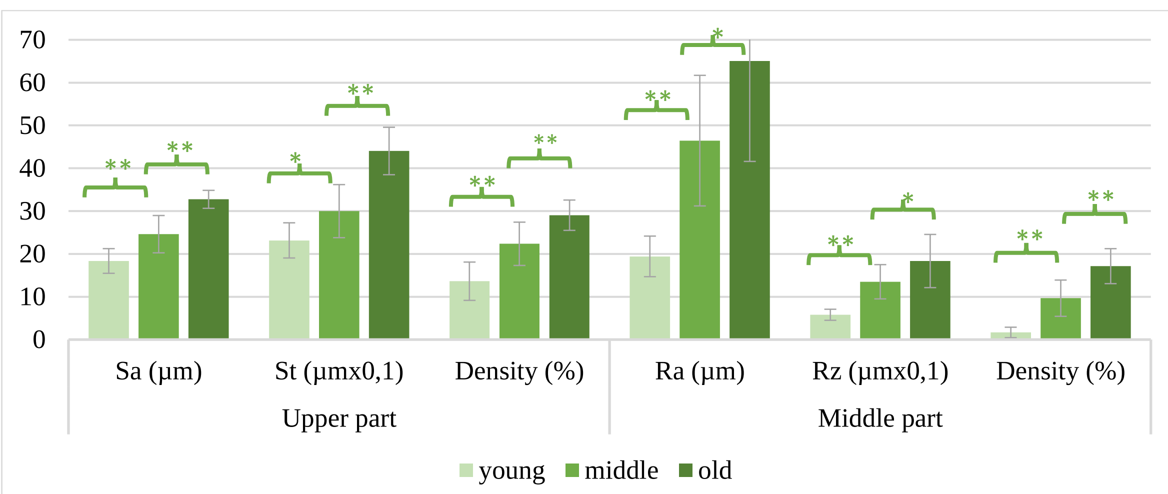


Figure 4: Skin topography parameters obtained with AEVA on upper and middle part of the knees. The figure present parameters and the comparison between young, middle and old age categories. *$p<0.05$; ** $p<0.01$*

Skin elasticity – Cutometer®

Classical R parameters Ua/Uf (R2), Ur/Ue (R5), Uv/Ue (R6), Ur/Uf (R7) and "skin fatigue" (R4-R1) showed a significant correlation with age (Table 2). The area parameter F3 (skin fatigue) and the Q parameters Q1 (Overal elasticity), Q2 (elastic recovery) and Q3 (viscous recovery) also showed a significant correlation with age (Table 2). The coefficients R^2 were all above 0.44 and no differences were seen between upper and middle part of knees.

Table 2: Correlations between cutometer® parameters and age, ***p<0.01*

	R2		R5		R6		R7		R4-R1	
	Upper part	Middle part	Upper part	Middle part	Upper part	Middle part	Upper part	Middle part	Upper part	Middle part
Correlation coefficient (R^2)	0,5764**	0,5044**	0,5865**	0,6915**	0,4962**	0,4785**	0,6558**	0,75**	0,6197**	0,4423**
	F3		**Q1**		**Q2**		**Q3**			
	Upper part	Middle part	Upper part	Middle part	Upper part	Middle part	Upper part	Middle part		
	0,6394**	0,4665**	0,5959**	0,5884**	0,6438**	0,726**	0,7231**	0,8538**		

For next results, only upper part is presented as the results are very similar to those obtained with middle part.

The difference between "young", "middle" and "old" was significant for all the parameters except for R6 (Fig. 5).

R2, R5 and R7, ratios of recovery / extension are decreased in middle age category compared to young category and in old category compared to middle category. R6, ratio of viscosity increased in middle age category compared to young category but only showed a tendency to decrease in old category compared to middle category ($p=0.1$). R4-R1 and F3, related to skin fatigue are both deteriorated in middle age category compared to young category and in old category compared to middle category. The Q parameters also showed the same pathway.

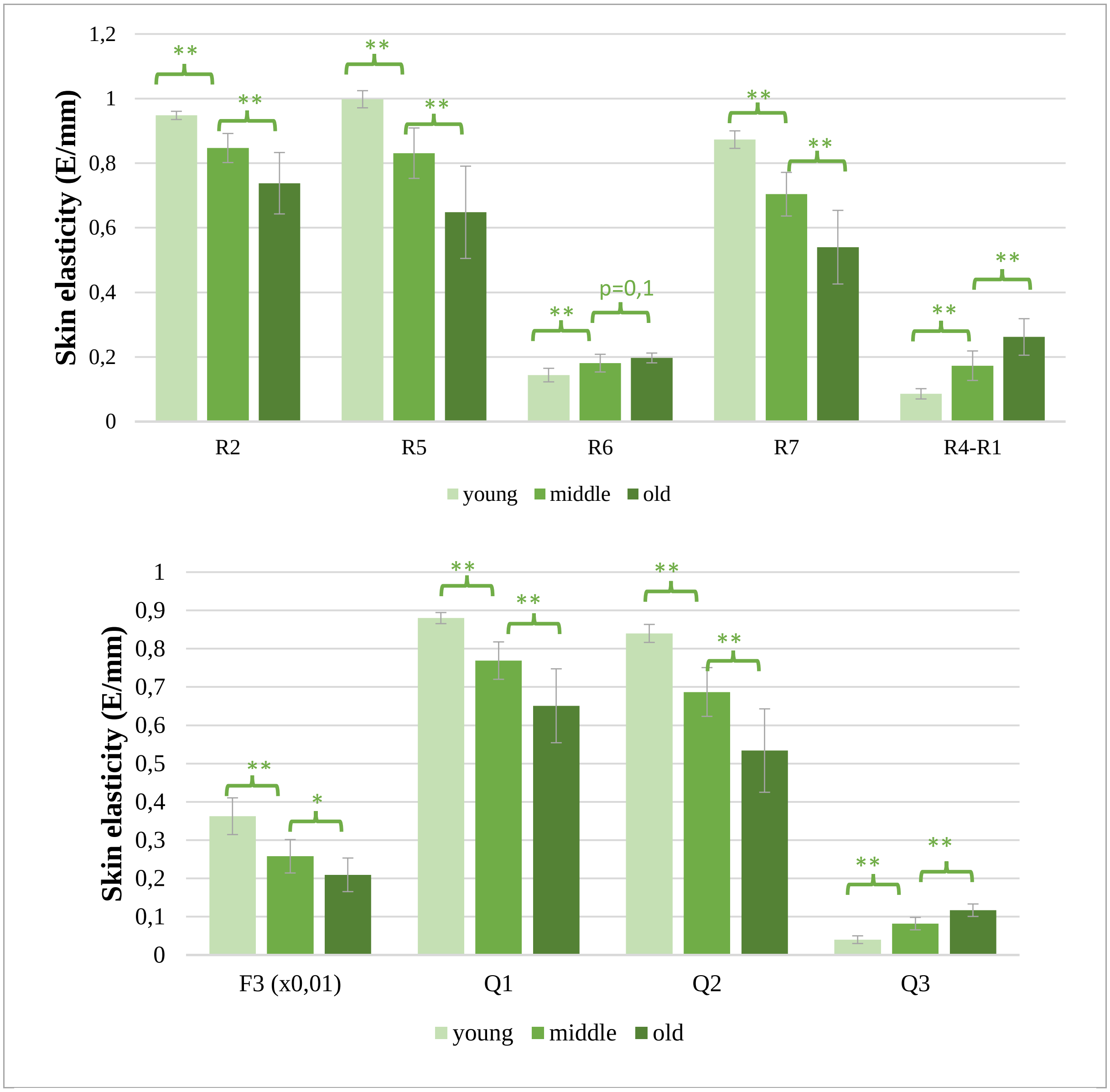


Figure 5: Skin elasticity parameters obtained with Cutometeter® on upper part of the knees. The figure present cutometer parameters and the comparison between young, middle and old age categories. **p<0.05; ** p<0.01*

Skin elasticity – ElastiMeter®

The use of ElastiMeter® on middle part of the knee did not provide reproductible values, very important variations were observed between the three measures, so, we considered this site as non-relevant for ElastiMeter® measurement.

Moreover, 5 volunteers failed to have relevant right-left measurements, they were removed from analysis. In consequence, only upper part results on 25 volunteers are presented here.

The Instant Skin Elasticity showed negative correlation with age (Fig. 6) but this correlation remains low with a correlation coefficient $R^2 = 0.2449$ ($p<0.05$).

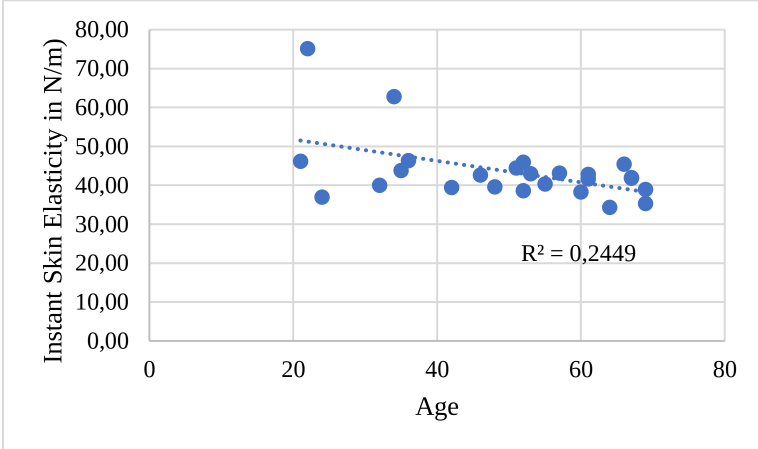


Figure 6: Skin elasticity parameter obtained with ElastiMeter® on upper part of the knees. The figure presents Elasticity *versus* age. *$p<0.05$

The difference between "young" and "middle" or "young" and "old" categories are almost significant, both with $p<0.07$ (Fig. 7). However, the comparison between "young" and "middle+old" panelists showed a significant difference with $p<0.05$.

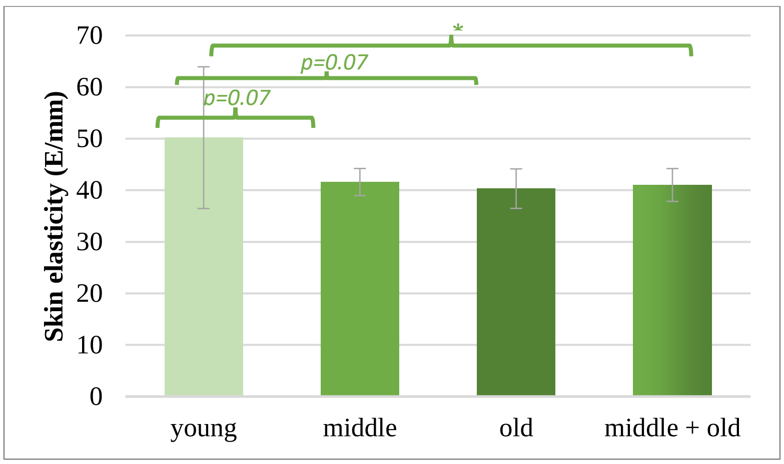


Figure 7: Skin elasticity parameter obtained with ElastiMeter® on upper part of the knees. The figure presents comparison between young, middle, old and middle+old age categories. *$p<0.05$

Skin density

Unfortunately, it was not possible to use the ultrasound probe in the middle part of the knee because of unflatten structures created by patella. Thus, the first part of the study consisted in comparing, on a few subjects, measurements performed both with 20 Mhz and 50 Mhz probes in the upper part of the knee. The 50 Mhz probe has provided more precise pictures, so, it was used for all the volunteers.

Visual observation of each echography and comparison amongst different volunteers failed to show any pattern or relationship with age, either in the density or the thickness of dermis. Thus, no quantification was done on pictures. Moreover, the Sub Epidermal Non-Echogenic Band (SENEB, [15]), that classically appears with age was not found here (Fig. 8).

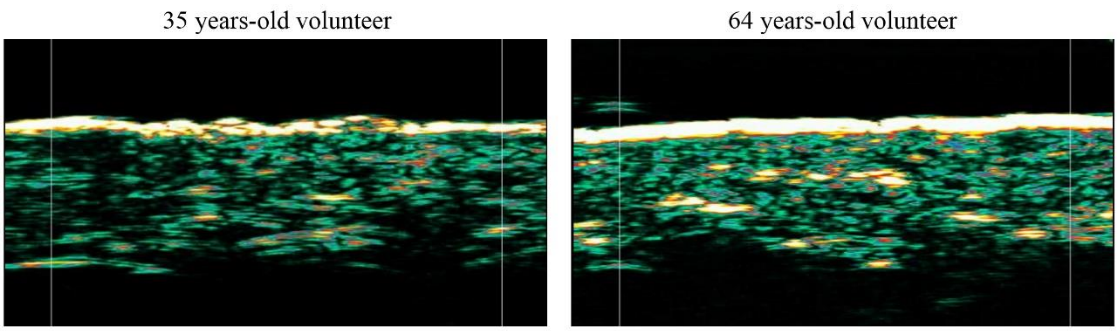


Figure 8: Example of ultrasound echography with a 50 Mhz probe

<u>Discussion.</u>

The setup of our photographic bench picture system has been realised after several steps involving shooting parameters but mostly position of the volunteer. The development and use of different systems to control this position allowed us to provide a very good reproducibility of our bench, even with aged people. This is crucial for demonstrating with pictures the efficacy of cosmetic products.

These and the creation of an atlas is useful to recruit people for any future ingredient efficacy testing. Depending what kind of relief must be studied – roughness, fine wrinkles, large wrinkles or sagging – this atlas allows to more accurately preselect volunteers according to age.

The study of skin relief with AEVA confirmed the previous results from Yoo *et al*. [1] with roughness parameters showing a correlation with age. In our study, the analysis of upper and middle part was made differently to consider the different nature of these 2 sites. The focus on micro-structure for upper part and on mid-structure for middle part was very efficient as we were able to see significant differences between young, middle and old age categories.

Regarding the skin elasticity with Cutometer®, we also confirmed the previous results from Yoo *et al.* [1] with a great number of parameters showing a correlation with age. Moreover, in our study, we evaluated results from upper and middle part of knees. We saw only small differences, but we noticed that the upper part of knee tends to be more elastic than the middle part of the knee skin (data not showed), this was correlated to our visual picture analysis.

A very important result was the differences found between young, middle and old age categories that proves the accuracy and good resolution of the Cutometer®. This device is obviously of interest in the case of efficacy testing.

New device ElastiMeter® was tried on the knees and gave mixed results. This partially confirmed previous results obtained on forearm by Viren *et al.* [14]. Herein the correlation with age and the difference between some age categories were seen, but we must emphasise the difficulties to use this equipment on middle part of knees and the obligation to remove some volunteers because of bad results. These technical and handling constraints could certainly be improved with longer training and a better and specific selection of volunteers with more uniform knees.

To our knowledge, no studies of knee dermis echogenicity have already been done and it was thus interesting to correlate surface appearance and dermis density. Regarding skin density with ultrasound echograph DermaScan® C, we must highlight the difficulty to use such probes on knees because of unflatten structures created by patella. Smaller probes could be of interest for this specific site. In the same way, the lack of clear SENEB in our acquisitions should be confirmed with another probe, even if it is likely because of lower sun exposure compared to SENEB found on face or external forearm.

Conclusion.

A photographic and a fringe projection bench have been set up to obtain accurate and reproducible acquisitions of knees. This allowed us to build up a 2D and 3D atlas with six age categories that clearly show the ageing of knees and its unsightly aspect. Roughness parameters and feature density calculated with fringe projection system demonstrate a positive high correlation with age for every part of the knee. Elasticity analysis with the Cutometer® has also provided a high correlation with age for the same sites. ElastiMeter® was only possible on upper part of the knee and has given low correlated results with age.

On the other hand, ultrasound device did not show any clear differences in the skin dermis density of young or more aged volunteers.

In conclusion, we have confirmed that wrinkles and elasticity on the knees are age dependent. Amongst various quantification methods, fringe projection and Cutometer® parameters are the best to precisely quantify knee ageing whatever it is on relatively smooth upper part or heavy wrinkled middle part. These methods could also be applied for the cellulite evaluation above the knee.

Conflict of Interest Statement. NONE.

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