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## Clinical validation of a computer-based approach for the quantification of the skin aging process of women using in-vivo confocal microscopy.

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Reflectance confocal microscopy (RCM) is a powerful tool to visualize the skin layers at cellular resolution up to a depth of  $200\mu\text{m}$ . A semi-quantitative score of skin aging from RCM images has been previously published, requiring visual assessment of the images by experienced dermatologists [REF]. We have proposed new computer-based methods to automatically quantify the skin aging process on RCM images. This letter presents the results of a clinical study designed to validate the computer-based methods versus expert assessment of RCM skin images. The study was conducted according to Good Clinical Practices and followed the Helsinki convention. The protocol was approved by the internal ethics comity of the Modena and Reggio Emilia Hospital, Italy. We enrolled 160 healthy women from 4 different ethnic groups (Caucasian, Hispanic, African and Asian). RCM images were acquired on the cheek using a near-infrared reflectance confocal laser scanning microscope. Clinical annotations were evaluated by an expert dermatologist to assess the following aging sign: epidermal honeycomb pattern; shape of the dermal papillae; organization of the dermal fibers (Fig1).

The epidermal honeycomb pattern was annotated as either regular or irregular. The proposed algorithm calculates the *percentage of regular cells* and the *average size of regular regions* to provide scores at the epidermal layer.

The shape of the dermal papillae was clinically annotated as ringed, poly-cyclic or both. This shape was computationally characterized by local and topological descriptors, through their *number*, *compactness* and *elongation*. The last two tend to 1 for a perfect circle. Sixty seven out of 160 subjects were used for the validation of the DEJ algorithm, as they presented only one visible type of pattern, i.e. ringed or poly-cyclic. Most subjects with visible dermal papillae had a dark photo-type.

Finally, collagen fibers type were manually annotated as being reticulated or coarse. The machine learning approach developed calculates the *percentage of reticulated fibers* in an image and classify the images as reticulated or coarse.

For each skin layer, experts' clinical annotations defined two populations. Means of distributions of the *algorithm parameters* were compared between them with Student's or Mann-Whitney tests ( $p < 0.05$ ), depending on the result of the normal distribution test (Shapiro normality test). Random Forest classifiers were used to create a predictive model for clinical annotations from the computer-based descriptors. The accuracy, sensitivity and specificity of these models were computed.

Regarding the epidermal layers, no statistical difference is found in the *percentage of regular cells* between the two populations, i.e. honeycomb patterns annotated as regular and irregular. On the contrary, the *average size of regular regions* is significantly higher among regular honeycomb patterns. The clinical annotation prediction achieves an average precision score of 80% while the sensitivity and specificity scores are slightly above 80%, **see Table 1**.

The number, mean compactness and elongation of dermal papillae are significantly higher among ringed dermal papillae than among poly-cyclic dermal papillae. We achieve 83%, 76% and 81% for respectively the global accuracy, sensitivity and specificity (Table 1).

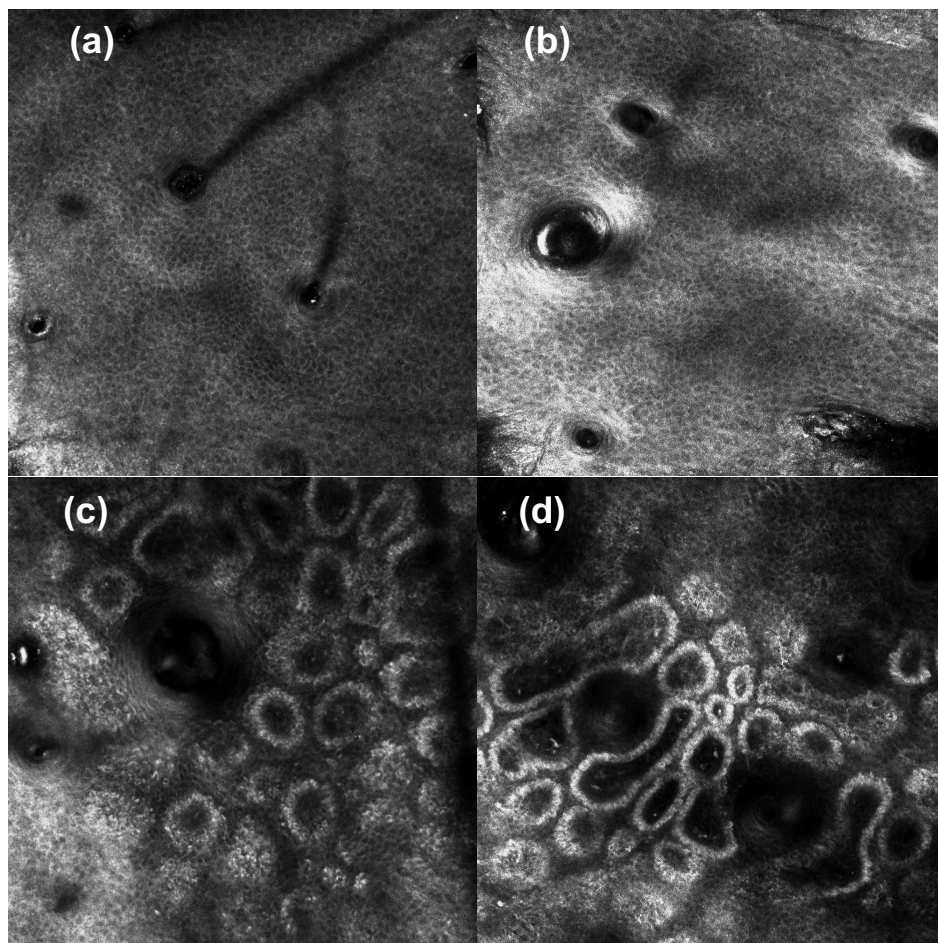
As for the collagen fibers, the percentage of reticulated fibers among is significantly higher in the reticulated population. We achieve a specificity of 89%.

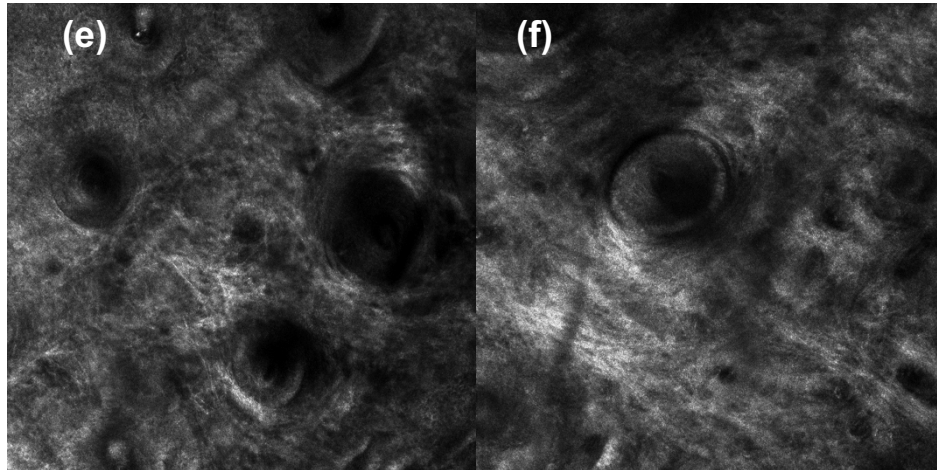
Computer classification of the main skin parameters, as observed in RCM, showed good correlation with the expert evaluator. To our knowledge, these are the first results comparing a computer-based approach to dermatologists' approach for the assessment of skin aging using in vivo confocal microscopy.

Skin Layer	Algorithm output	Mean of distributions for each population, i.e. clinical annotation	Clinical annotation prediction scores		
			Accuracy	Sensitivity	Specificity
Epidermis	Percentage of regular cells	Regular / Irregular $0,36 \pm 0,08 / 0,32 \pm 0,07$	80	81	81
	Average size of regular regions	Regular / Irregular $6.6 \pm 3.3 / 5.2 \pm 3.1$			
DEJ	Number of dermal papillae	Cyclic / Poly-cyclic $24.9 \pm 4.9 / 22 \pm 4.7$	83	76	81
	Mean compactness of dermal papillae [0,1]	Cyclic / Poly-cyclic $0.45 \pm 0.01 / 0.43 \pm 0.02$			

	Mean elongation of dermal papillae [0,1]	Cyclic / Poly-cyclic $0.58 \pm 0.02 / 0.56 \pm 0.02$			
<b>Dermis</b>	Collagen fibers type (reticulated/coarse)	Reticulated / Coarse $0,66 \pm 0,22 / 0,38 \pm 0,17$	80	63	89

Table 1





**Figure 1:** First row: Epidermal aging. (a) The epidermis exhibits a regular honeycomb pattern (b) Irregular honeycomb pattern. Second row: DEJ aging. (c) Ringed dermal papillae (d) poly-cyclic dermal papillae. Third row: Collagen fibers types. (a) Reticulated fibers characterized by tiny reflective fibers orderly disposed forming web like structures, (b) coarse fibers composed by thick fibers grossly arranged into nets.