8th Anti-Ageing Skin Care Conference

29 - 30 November 2022
Royal College of Physicians, London

proderm

SGS

Dermal Ageing: Assessment of Collagen, Elastin and AGE in vivo

Stephan Bielfeldt

Agenda

- Intrinsic and extrinsic aging of the dermis
- Non invasive in-vivo methods to assess ageing of the dermis
  - In vivo assessment of collagen and elastin
  - In vivo assessment of AGE
- Summary

The Dermis: About 1 mm thick (Epidermis ~ 60 µm)

Composition: Mainly Protein Fibres in a Gel of 4% of Hyaluronic Acid

TABLE I

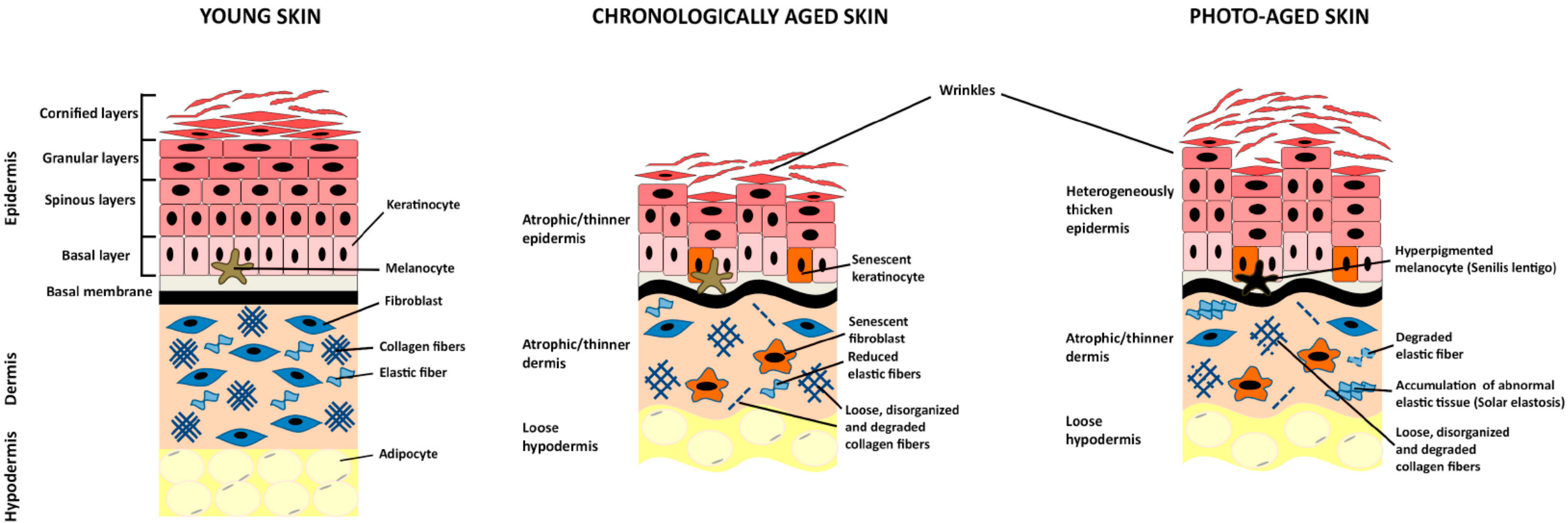
Fibrous proteins of human corium

	Male	Female
Collagen	75.9 ± 6.4* (47)	77.7 ± 5.9 (36)
Elastin	4.45 ± 1.4 (17)	3.95 ± 0.9 (10)

* Mean and standard deviation of the mean.

Weinstein, G. D., & Boucek, R. J. (1960). Collagen and elastin of human dermis. Journal of Investigative Dermatology, 35(4), 227-229.

Ageing of the Skin



Orioli, D., & Dellambra, E. (2018). Epigenetic regulation of skin cells in natural aging and premature aging diseases. *Cells*, *7*(12), 268.

Ageing and Photoageing of the Dermis

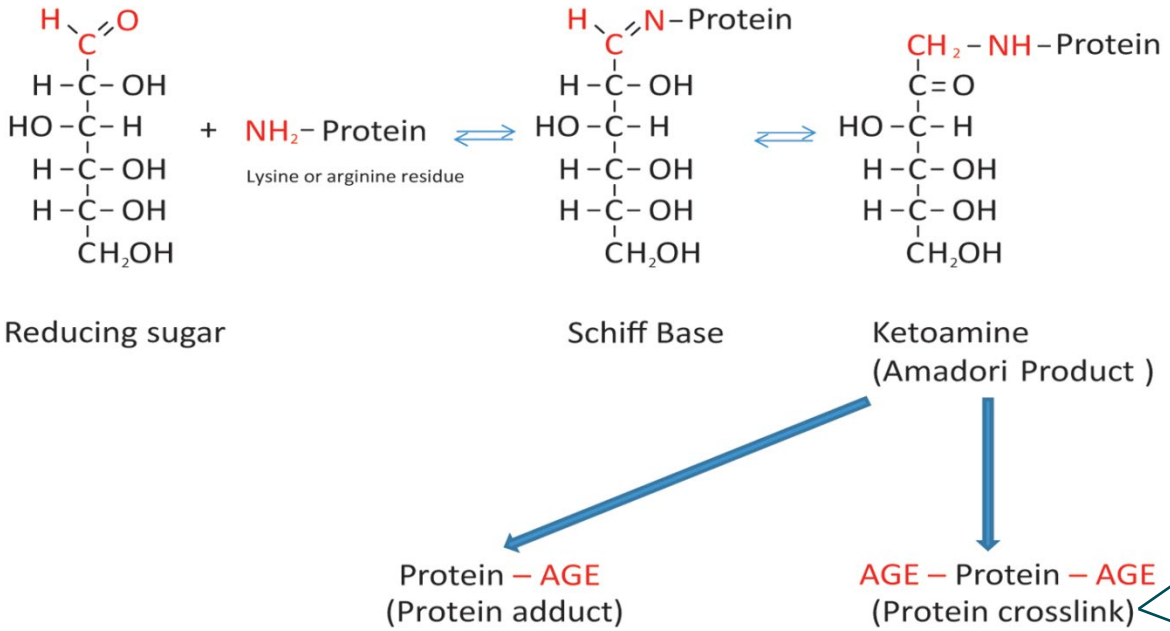
On cell level

- Senescence of fibroblasts
- Decrease in collagen synthesis
- Decrease in hyaluronic acid production

On tissue level

- MMP-1 formation by extrinsic stress (collagen cleavage)
- Degradation of collagen and elastin (formation of deep wrinkles)
- Deposition of dysfunctional elastin (solar elastosis)
- Thinning of the dermis (atrophy)
- Glycation of proteins (yellowish skin discoloration)

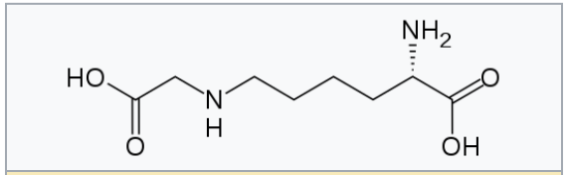
What are Advanced Glycation End-products (AGE)?



Maillard reaction: Reactive carbonyl groups of a reducing sugar react with free amino groups from proteins to form a Schiff base => Amadori product => AGE – protein

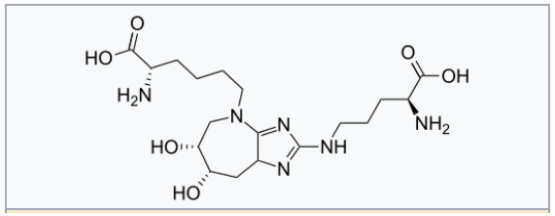
Marker molecules

N(6)-Carboxymethyllysine



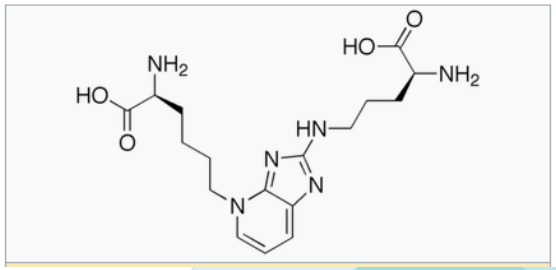
https://en.wikipedia.org/wiki/N(6)-Carboxymethyllysine

Glucosepane



https://en.wikipedia.org/wiki/Glucosepane

Pentosidine

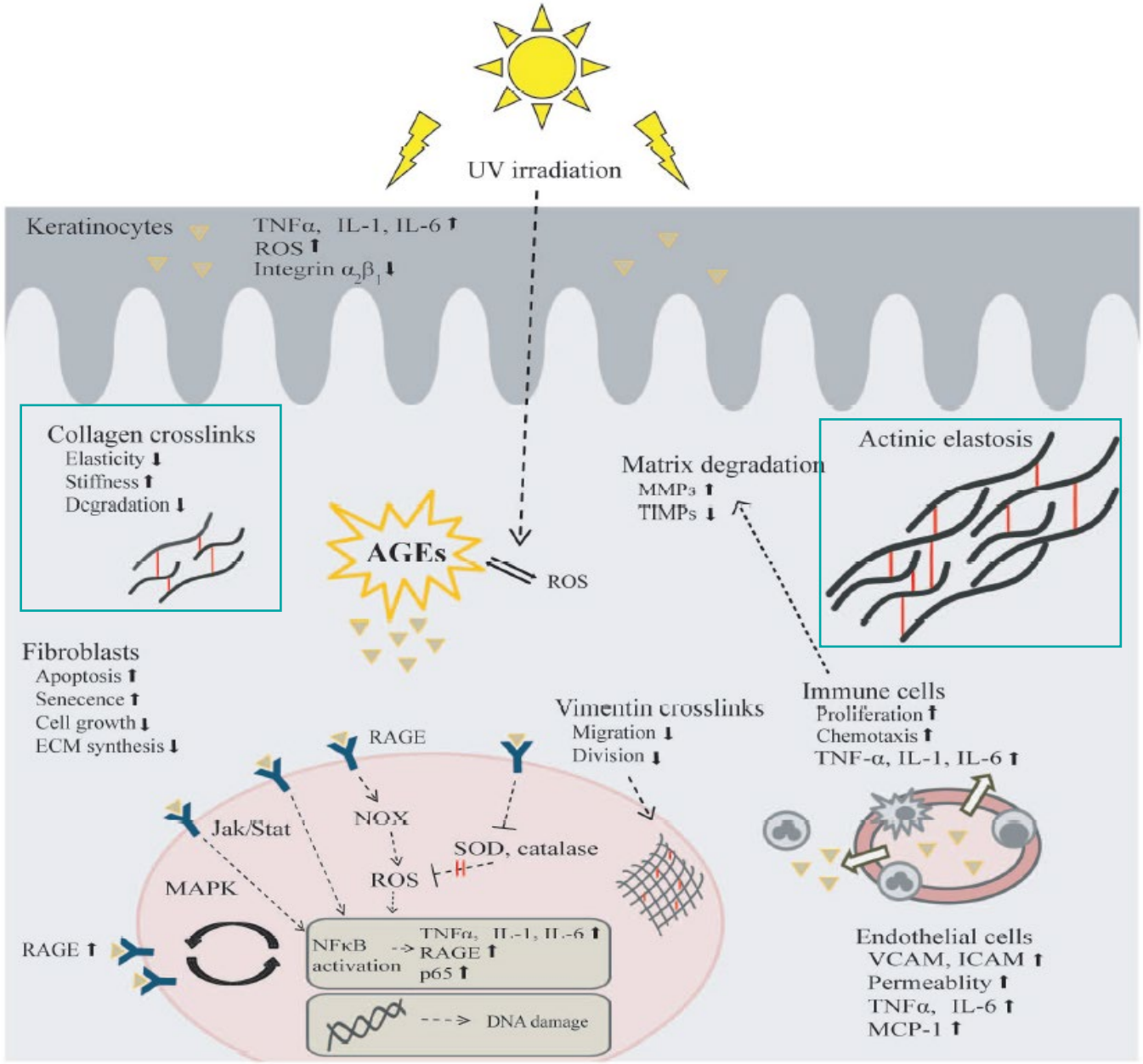


https://en.wikipedia.org/wiki/Pentosidine

Fluorescent

Gkogkolou, P., & Böhm, M. (2012). Advanced glycation end products: Key players in skin aging?. Dermato-endocrinology, 4(3), 259-270.

Effects of AGEs on Skin

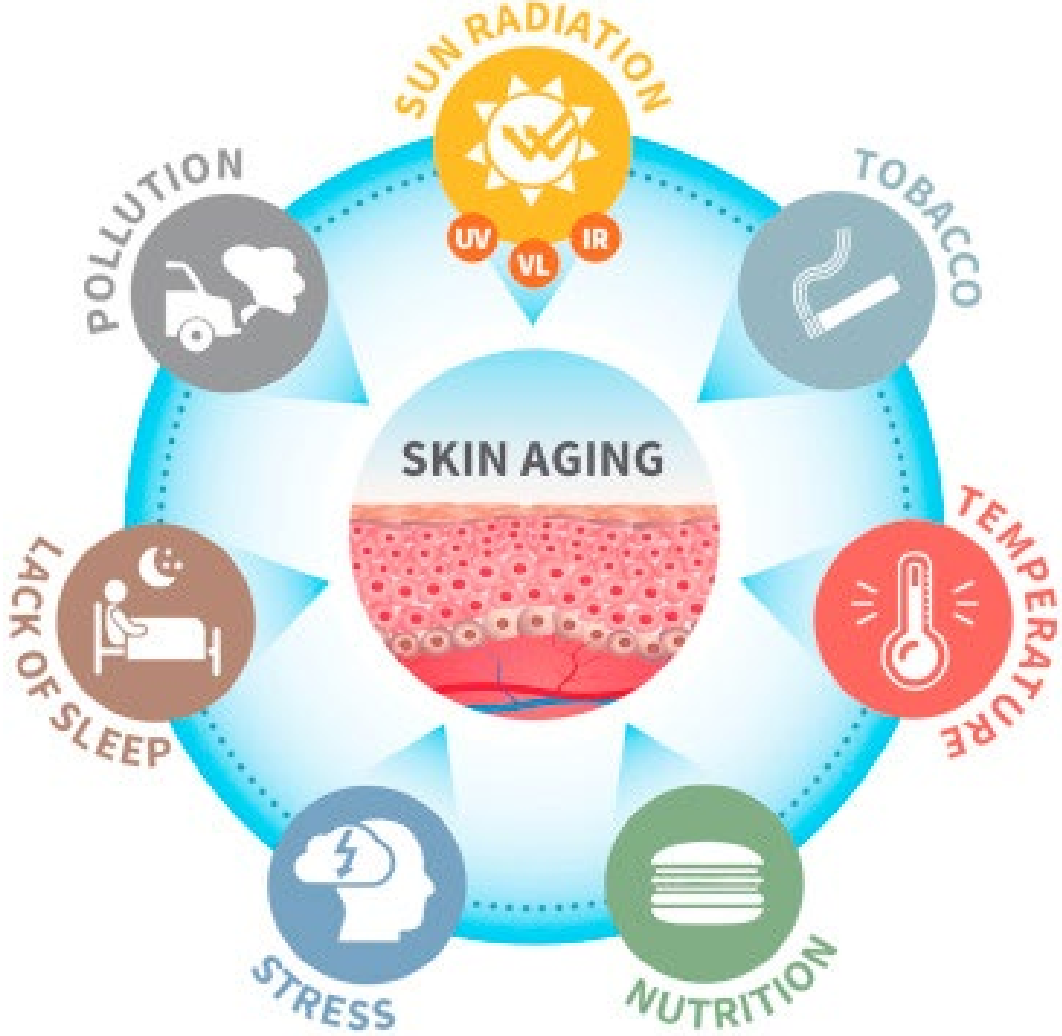


- Diabetes is an important aggravating disease but AGEs are also found in aged and photoaged skin
- ROS from sunlight and AGE reinforce each other in their deleterious effects
- AGEs decrease cell proliferation, increase senescence/apoptosis, induce oxidative stress and are proinflammatory
- AGEs induce collagen crosslinking and contribute to solar (actinic) elastosis

Gkogkolou, P., & Böhm, M. (2012). Advanced glycation end products: Key players in skin aging?. Dermato-endocrinology, 4(3), 259-270.

Extrinsic Ageing

The Skin Ageing Exposome: Causes are Manifold



Krutmann, J., Bouloc, A., Sore, G., Bernard, B. A., & Passeron, T. (2017). The skin aging exposome. Journal of dermatological science, 85(3), 152-161.

Visible Signs of Intrinsic and Extrinsic Ageing

Periorbital Wrinkles: Intrinsic ageing and photoageing

Wrinkles: Intrinsic ageing and on left face side

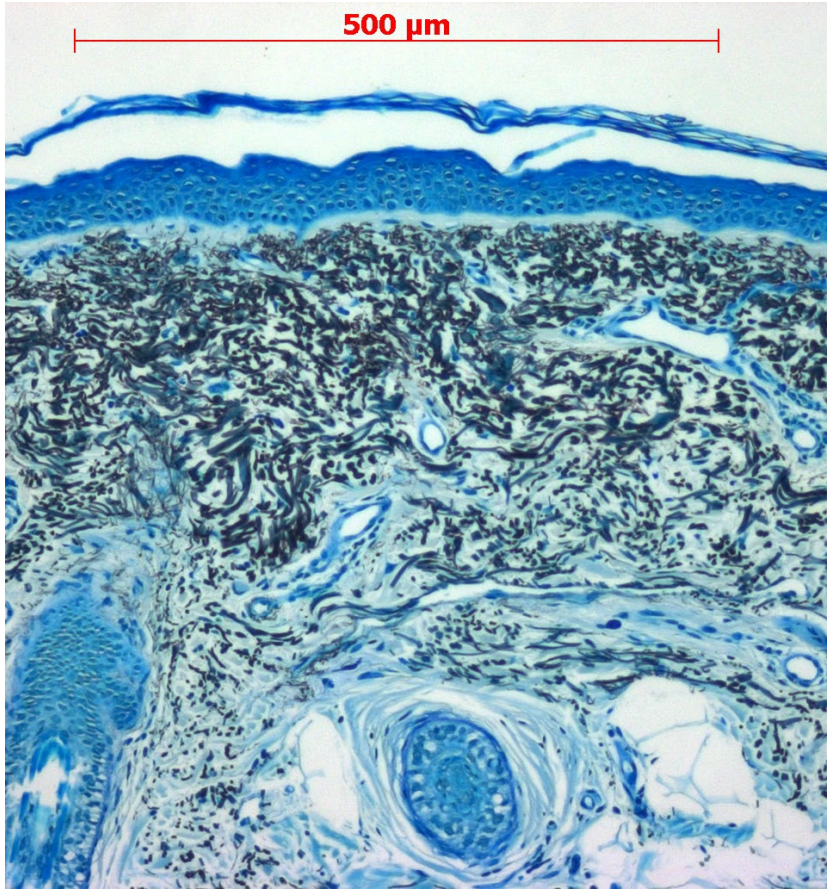
Photoageing on the right face side

Gordon, J. R., & Brieva, J. C. (2012). Unilateral dermatoheliosis. New England Journal of Medicine, 366(16), e25. Photograph under license: CC BY-NC-ND 4.0

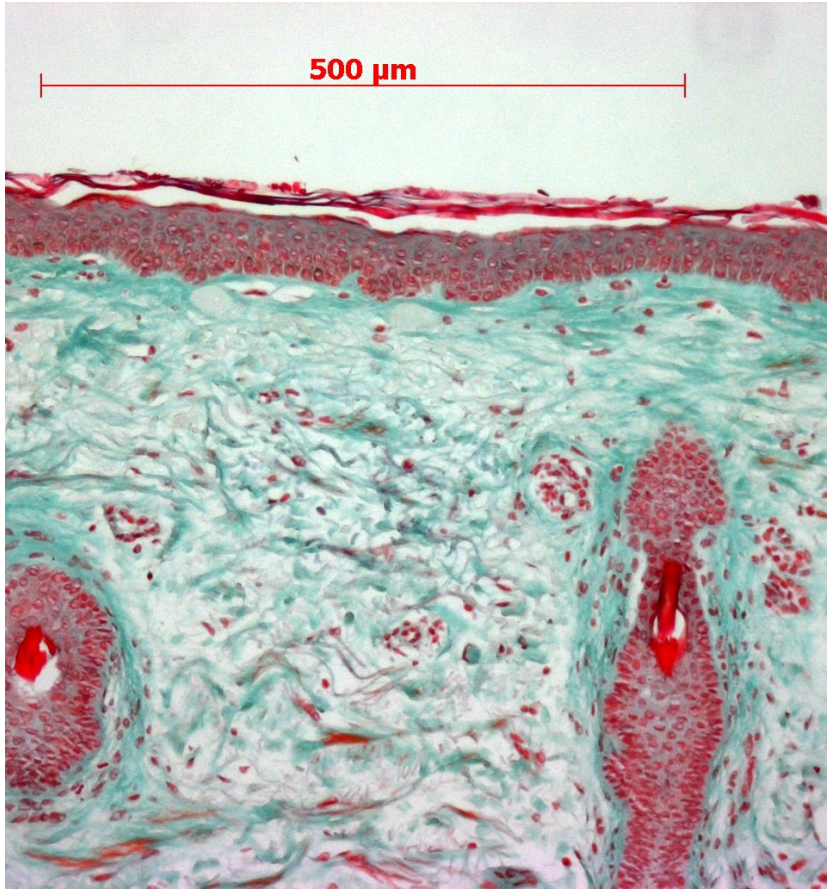
Agenda

- Intrinsic and extrinsic aging of the dermis
- Non invasive in-vivo methods to assess ageing of the dermis
  - In vivo assessment of collagen and elastin
  - In vivo assessment of AGE
- Summary

Invasive and ex vivo: Stainings for Collagen and Elastin



Orcein staining



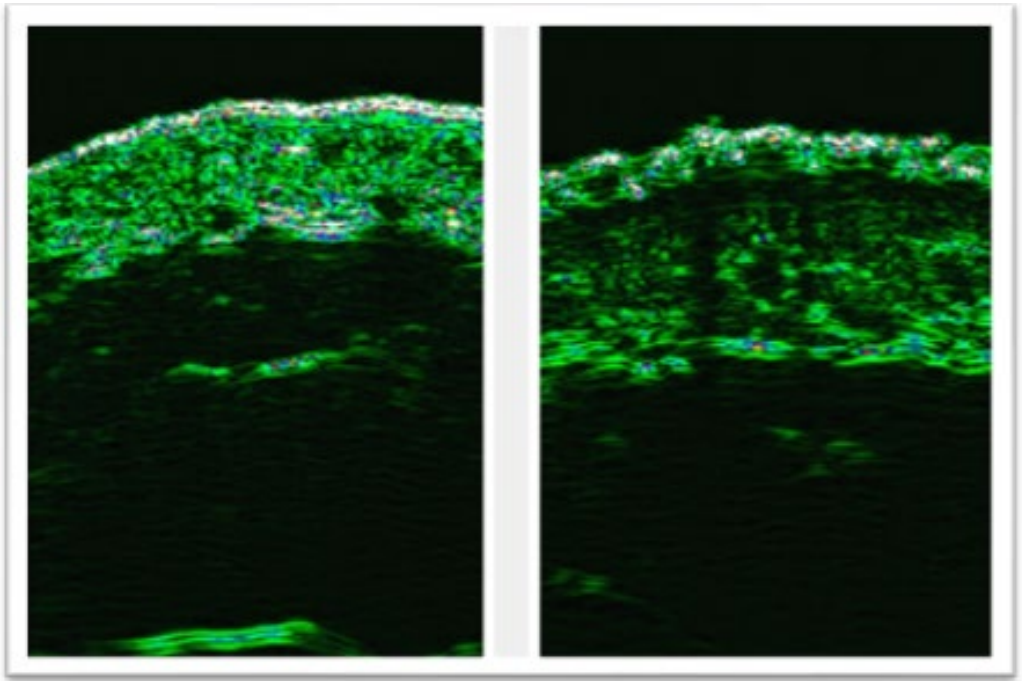
Masson-Goldner staining

- Orcein staining (black and blue): Elastic fibers in black
- Masson-Goldner staining (red and green): Collagen fibres in green.

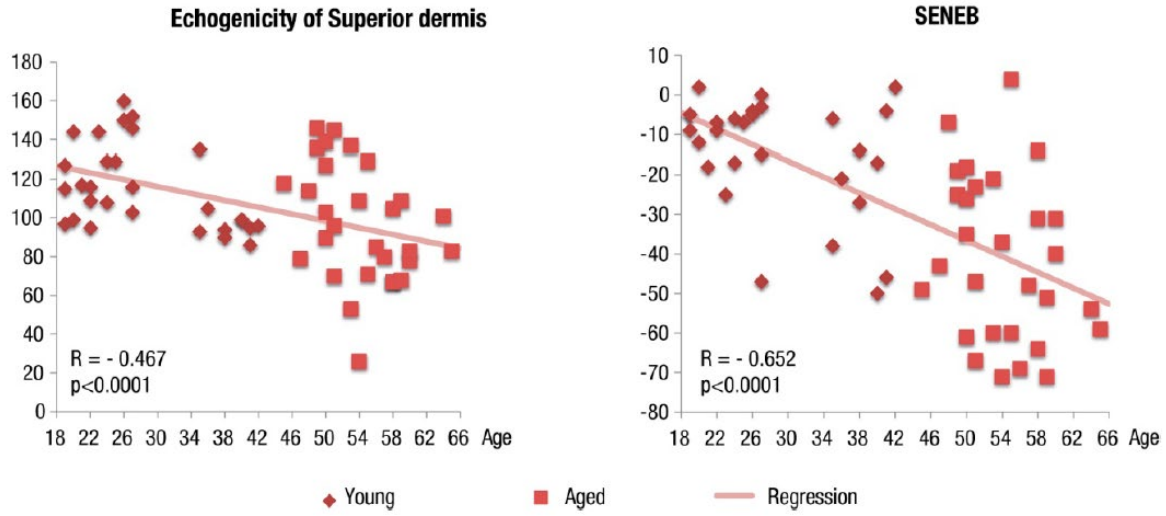
Bonta, M., Daina, L., & Muţiu, G. A. B. R. I. E. L. A. (2013). The process of ageing reflected by histological changes in the skin. *Romanian journal of morphology and embryology= Revue roumaine de morphologie et embryologie*, *54*(3 Suppl), 797-804.

22 MHz-Ultrasound Measurement on aged Skin

Sub Epidermal Non Echogenic Band (SENEB) due to Degraded Fibres in the Dermis



Normal skin (outer forearm) Photoaging: Echo poor region (SENEB) below epidermal echo



Pittet, J. C., Freis, O., Vazquez-Duchêne, M. D., Périé, G., & Pauly, G. (2014). Evaluation of elastin/collagen content in human dermis in-vivo by multiphoton tomography—variation with depth and correlation with aging. Cosmetics, 1 (3), 211-221.

Confocal Reflectance Microscopy

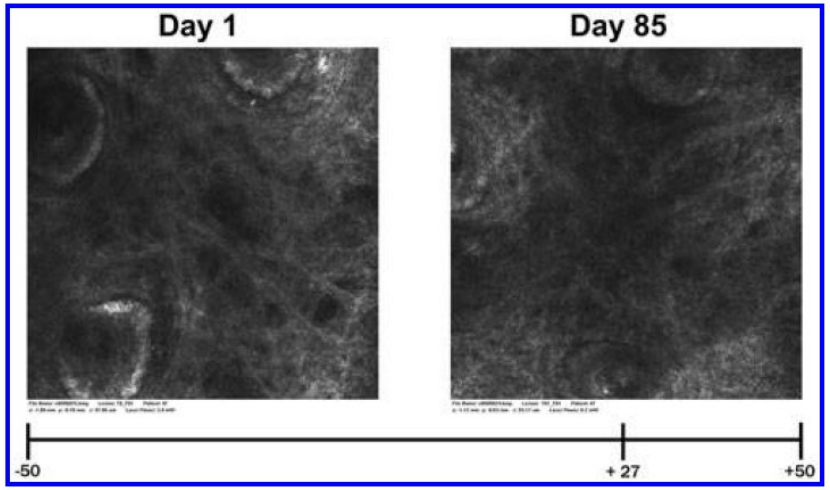
Visible Improvement of Collagen Structure Assessed in Blinded Image Grading on Horizontal Images of the Dermis

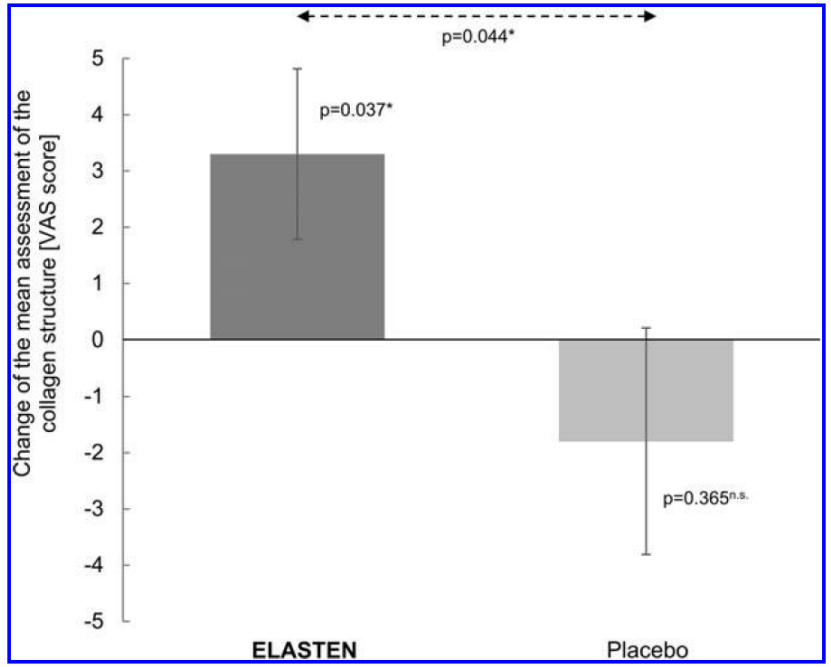
JOURNAL OF MEDICINAL FOOD
J Med Food 23 (2) 2020, 147–152
Mary Ann Liebert, Inc., and Korean Society of Food Science and Nutrition
DOI: 10.1089/jmf.2019.0197

A Dermonutrient Containing Special Collagen Peptides Improves Skin Structure and Function: A Randomized, Placebo-Controlled, Triple-Blind Trial Using Confocal Laser Scanning Microscopy on the Cosmetic Effects and Tolerance of a Drinkable Collagen Supplement

Sabrina Laing, Stephan Bielfeldt, Carolin Ehrenberg, and Klaus-Peter Wilhelm

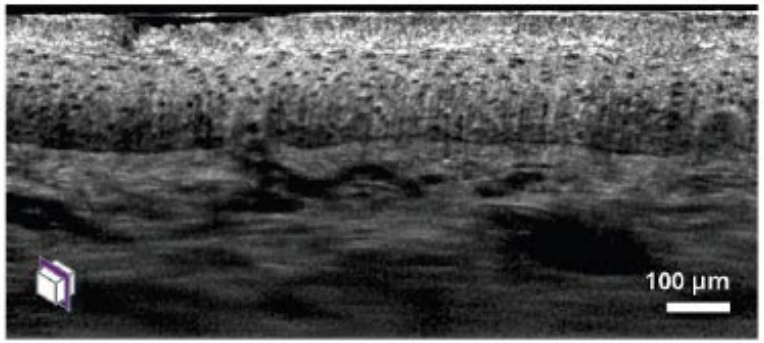
proDERM Institute for Applied Dermatological Research, Schenefeld-Hamburg, Germany.



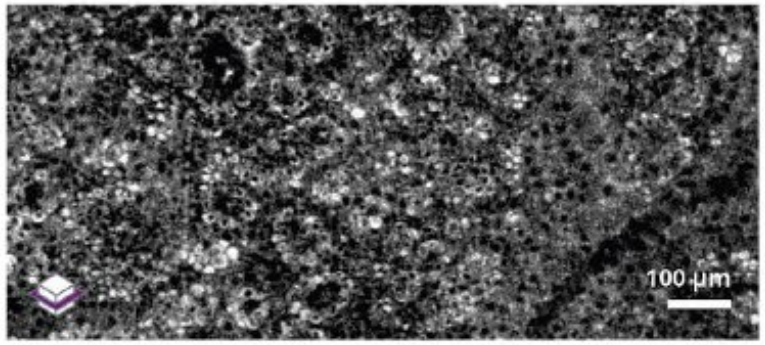


LC-OCT – Imaging of Epidermis, Papillary and Reticular Dermis down to ~ 400 µm; Resolution (x, y, z) ~ 1 µm

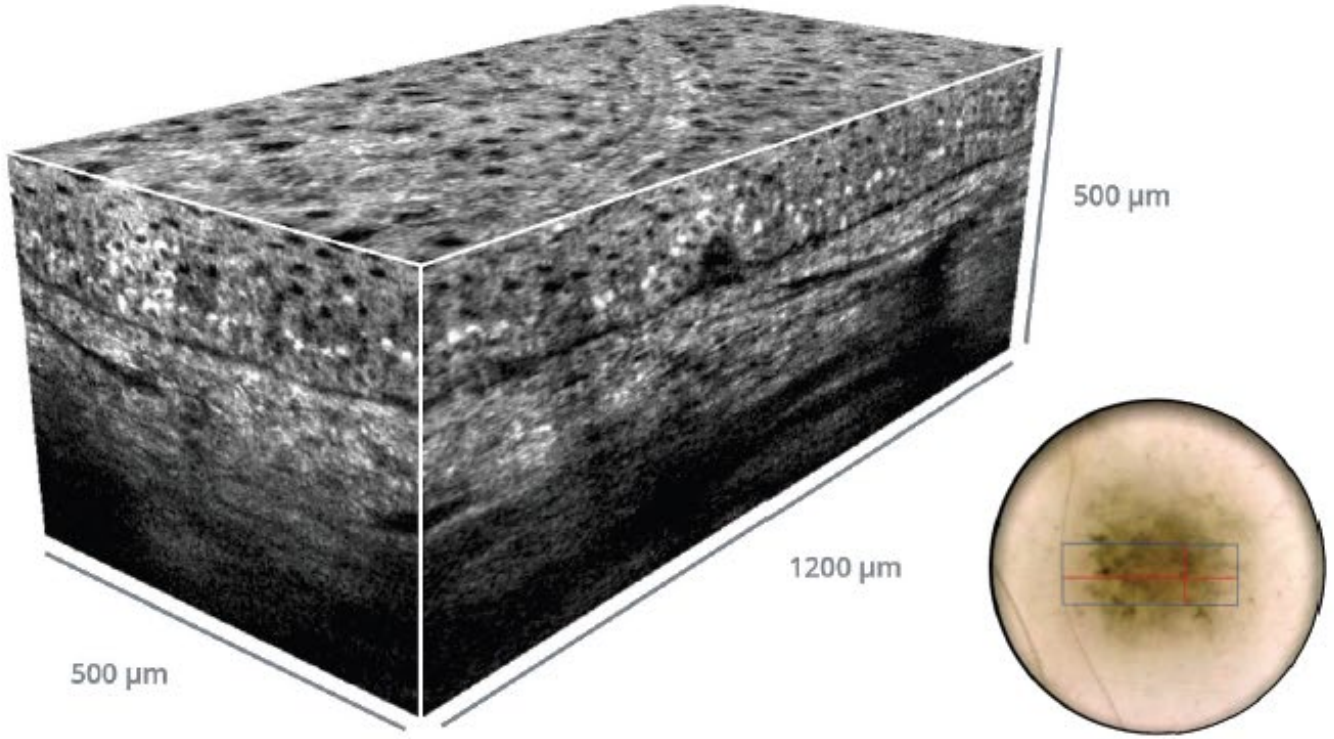
Vertical live mode



Horizontal live mode



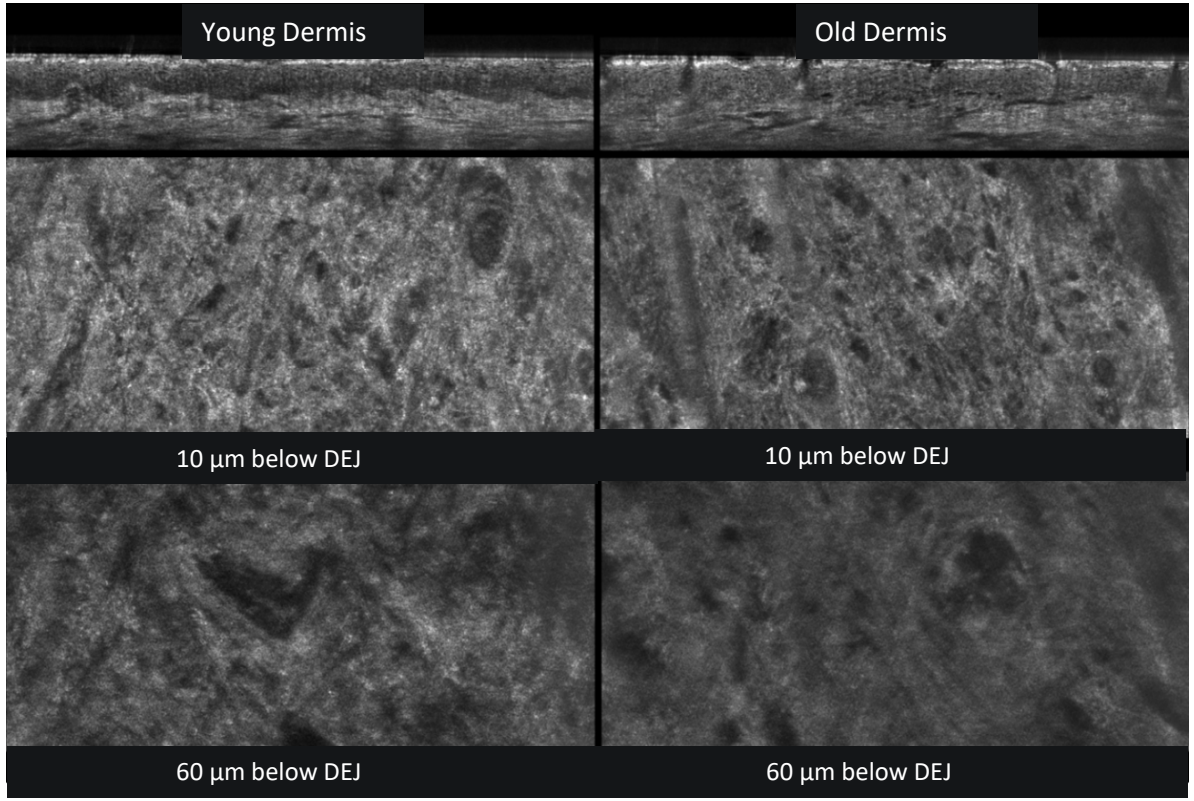
3D stack with isotropic cellular resolution

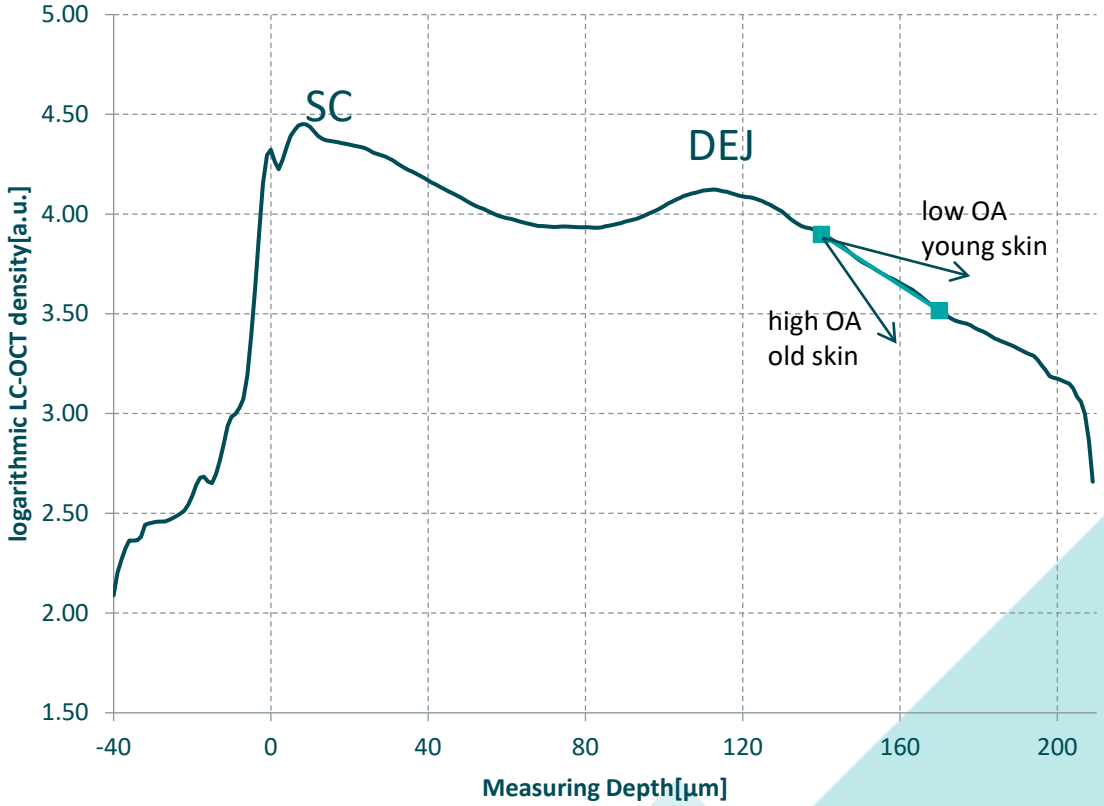


Imaging of an age spot

What is Optical Attenuation (OA)?

The LC-OCT Signal Becomes Weaker with Skin Depth

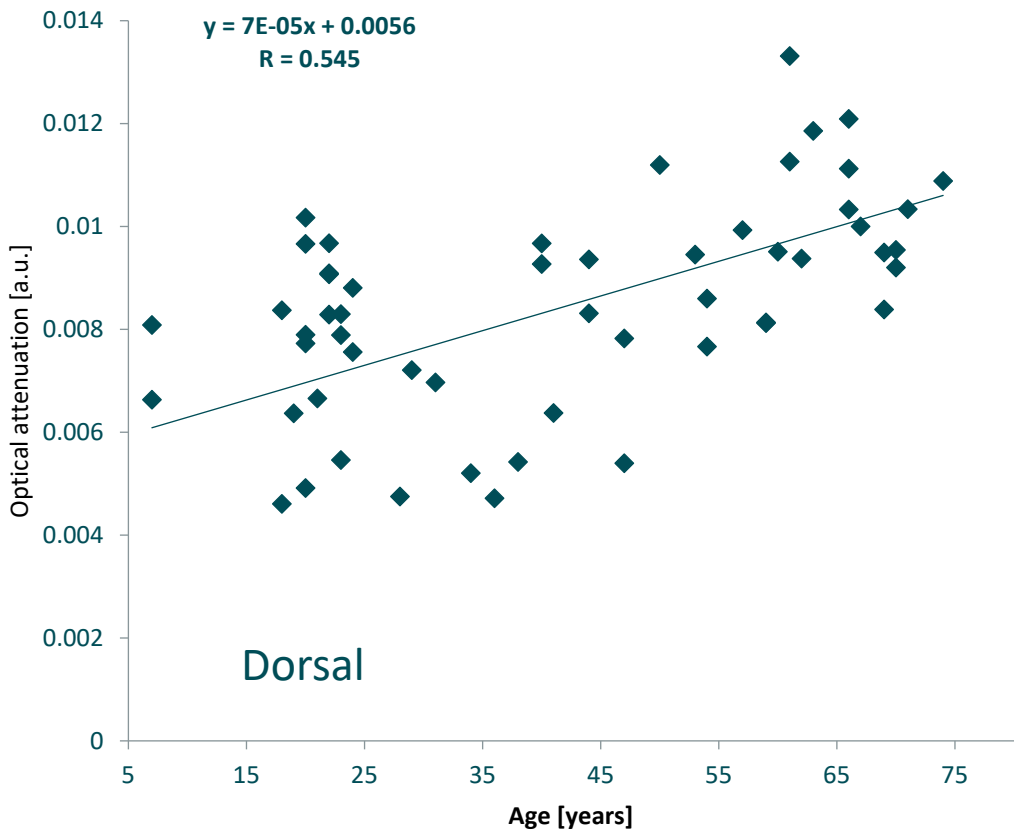


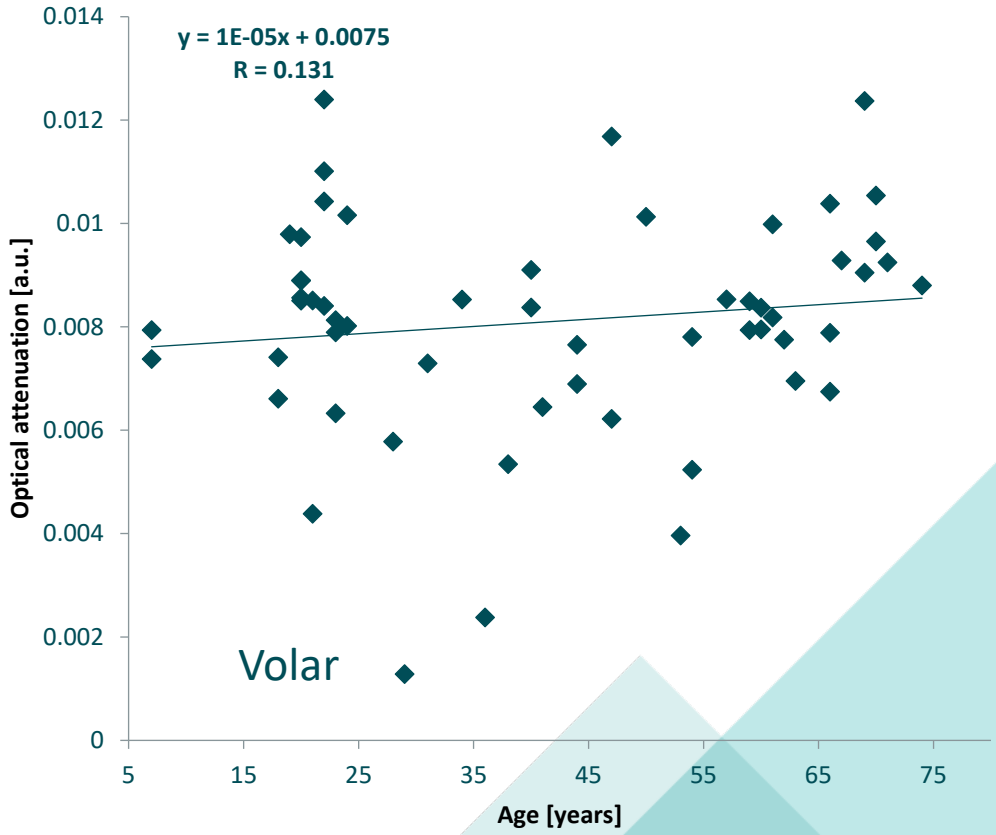


Optical Attenuation: The negative slope of the LC-OCT signal in the upper dermis
Young skin => high fibre reflection => low Optical Attenuation
Aged skin => low fibre reflection => high Optical Attenuation

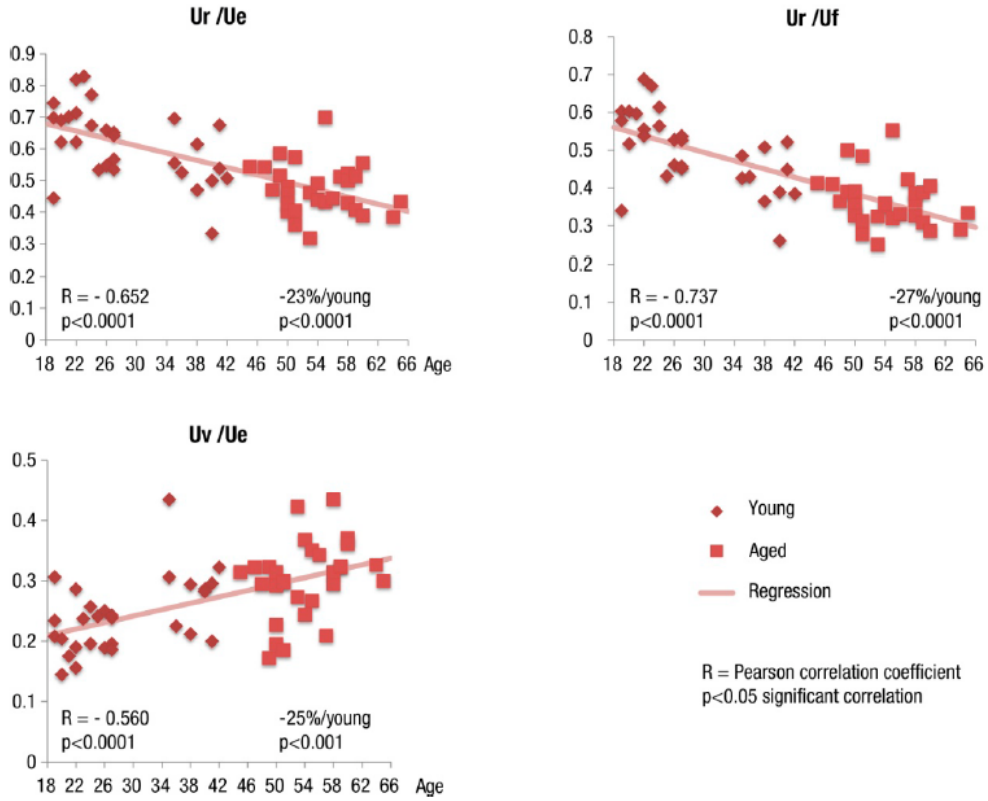
Correlation of dermal optical attenuation of dorsal and volar dermis with age

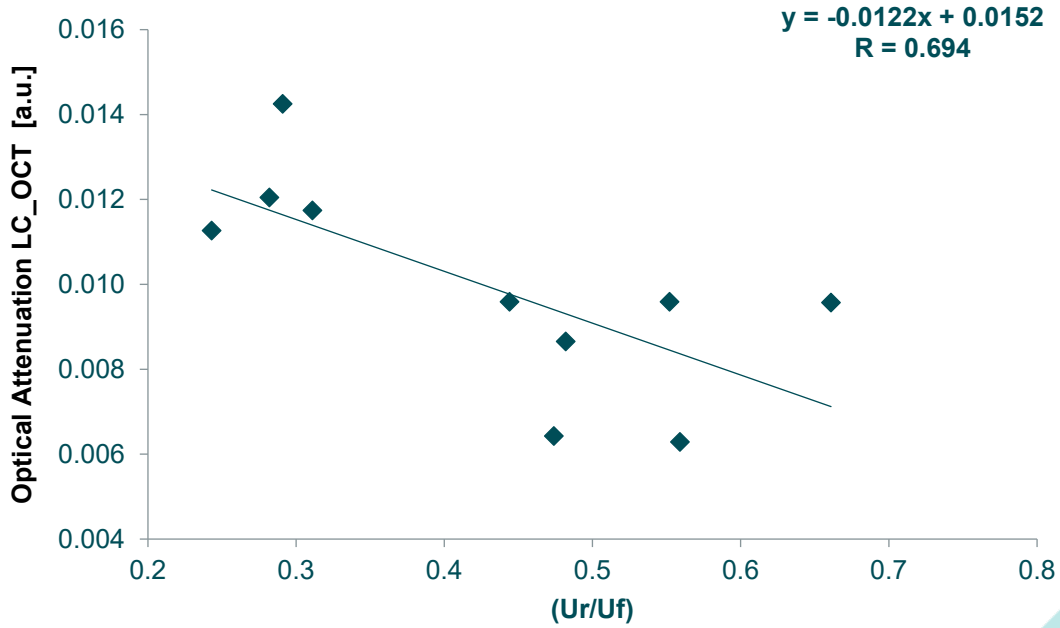
N = 57; R^2 = 0.297; depth 140 - 170 µm; analyzed from by LC-OCT data

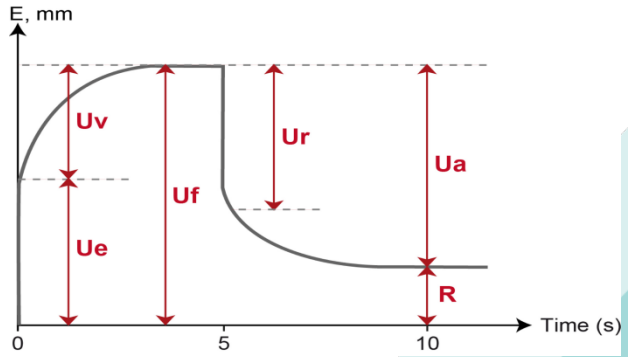




Skin Elasticity Measurement (Suction device)



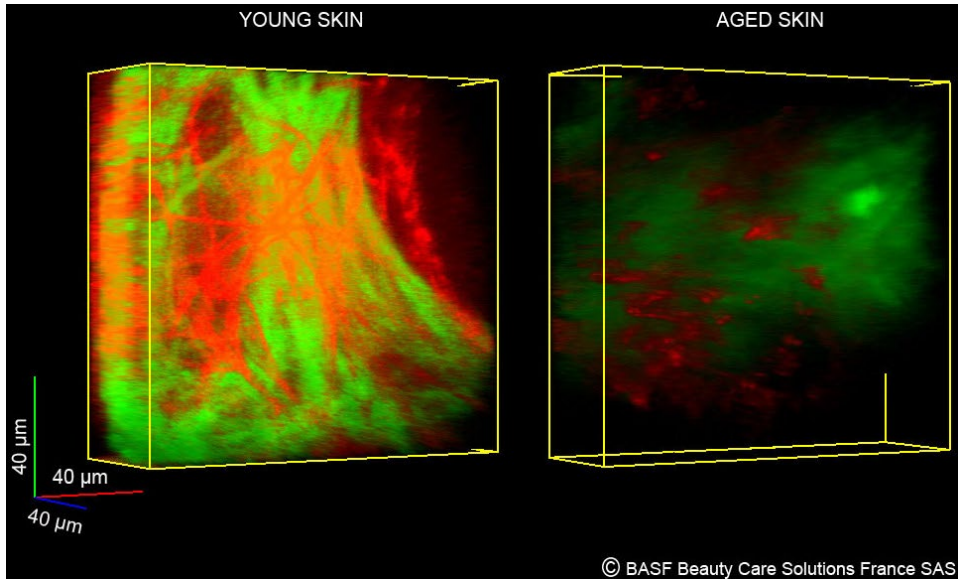




Cutometer® MPA 580, Courage+Khazaka Electronic GmbH, Köln, Germany

Pittet, J. C., Freis, O., Vazquez-Duchêne, M. D., Périé, G., & Pauly, G. (2014). Evaluation of elastin/collagen content in human dermis in-vivo by multiphoton tomography—variation with depth and correlation with aging. *Cosmetics*, *1* (3), 211-221.

Multiphoton Tomography of the Upper Dermis Imaging of Collagen and Elastin



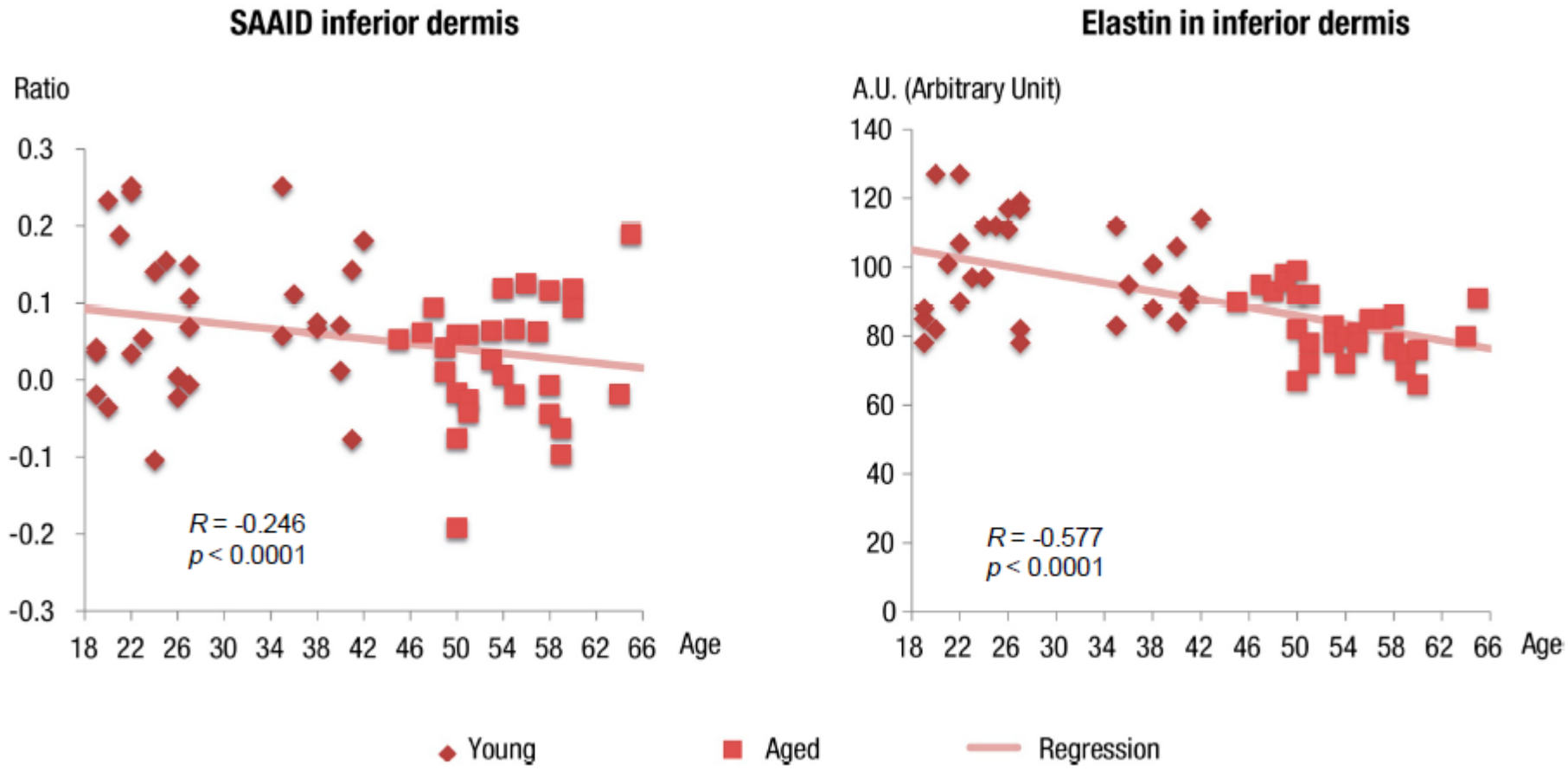
- Autofluorescence of elastin and collagen crosslinks (red color)
- Collagen Second Harmonic Generation (SHG)-signal (green color)

- Quantification Parameter: SHG-to-AF Aging Index of Dermis (SAAID)

- SAAID = (Intensity of Collagen – Intensity of Elastin)/(Inten,sity of Collagen + Intensity of Elastin)

Pittet, J. C., Freis, O., Vazquez-Duchêne, M. D., Périé, G., & Pauly, G. (2014). Evaluation of elastin/collagen content in human dermis in-vivo by multiphoton tomography—variation with depth and correlation with aging. *Cosmetics*, *1* (3), 211-221.

Multiphoton Tomography of the Upper Dermis Imaging of Collagen and Elastin



Pittet, J. C., Freis, O., Vazquez-Duchêne, M. D., Périé, G., & Pauly, G. (2014). Evaluation of elastin/collagen content in human dermis in-vivo by multiphoton tomography—variation with depth and correlation with aging. *Cosmetics*, *1* (3), 211-221.

Is Water Replacing Collagen in Ageing/Photoageing of the Dermis?

Received: 18 June 2020 | Accepted: 5 September 2020

DOI: 10.1111/srt.12948

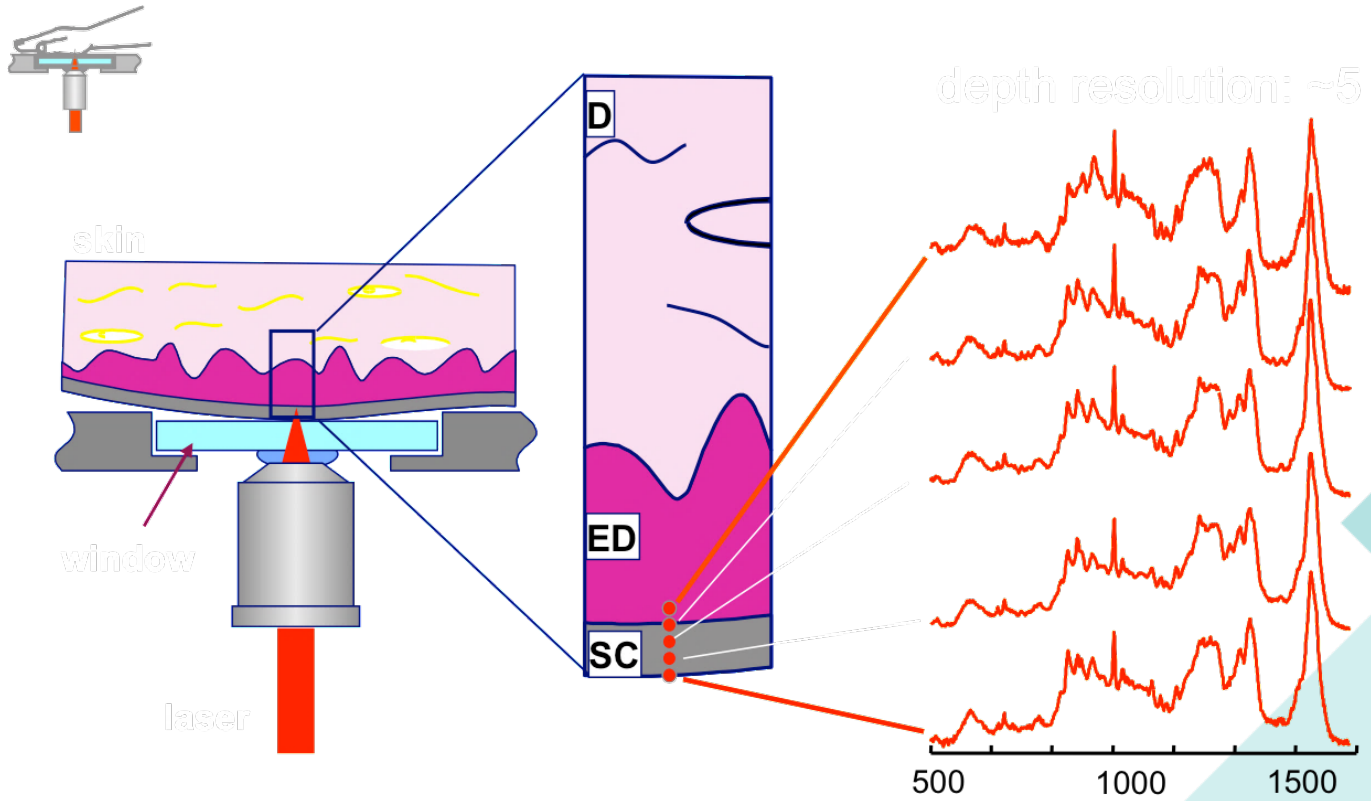
ORIGINAL ARTICLE

WILEY

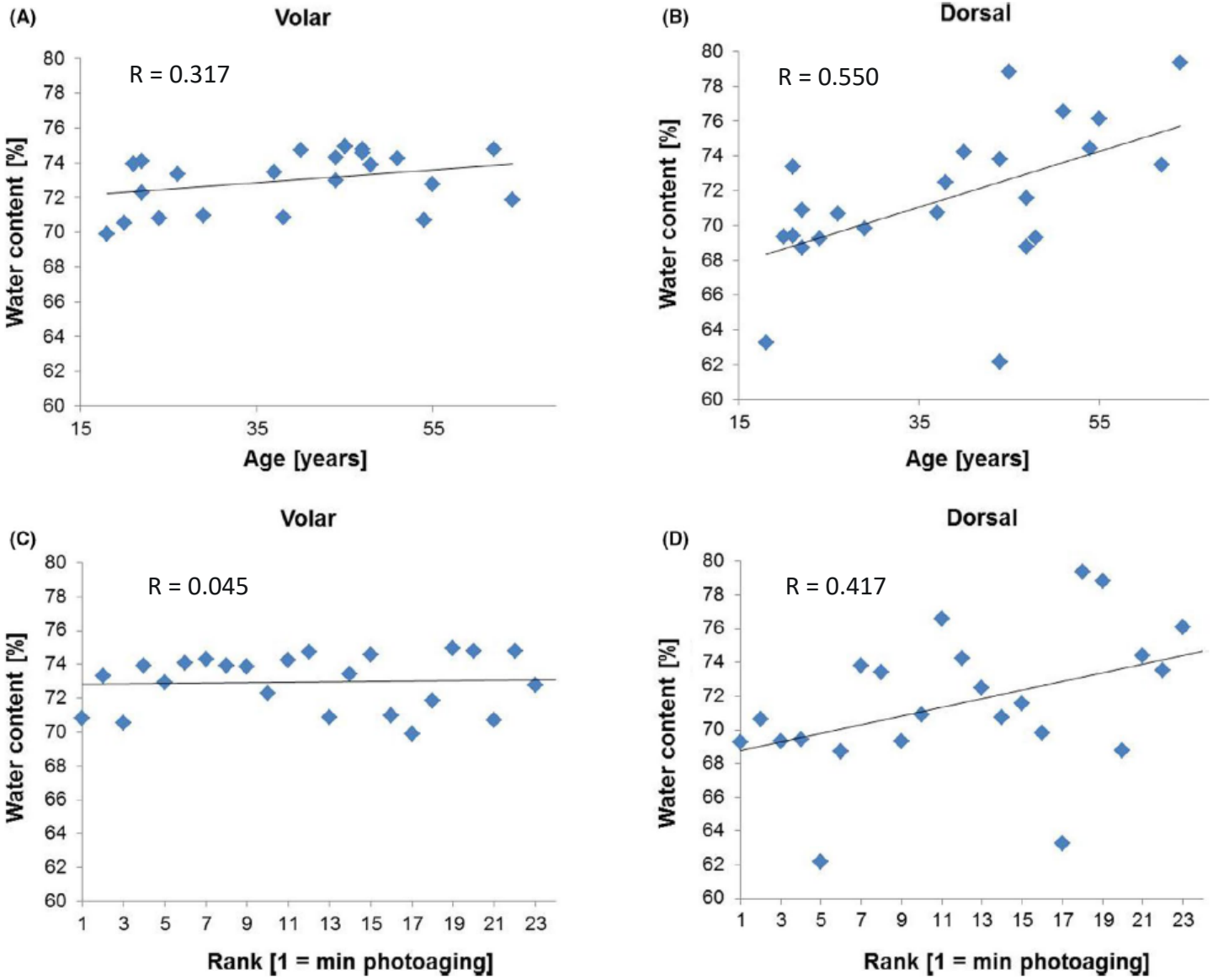
Measurement of dermal water content by confocal *RAMAN* spectroscopy to investigate intrinsic aging and photoaging of human skin in vivo

Ghaith Kourbaj | Stephan Bielfeldt | Matthias Seise | Klaus-Peter Wilhelm

Confocal Raman Microspectroscopy can Measure Water Content in the Upper Reticular Dermis



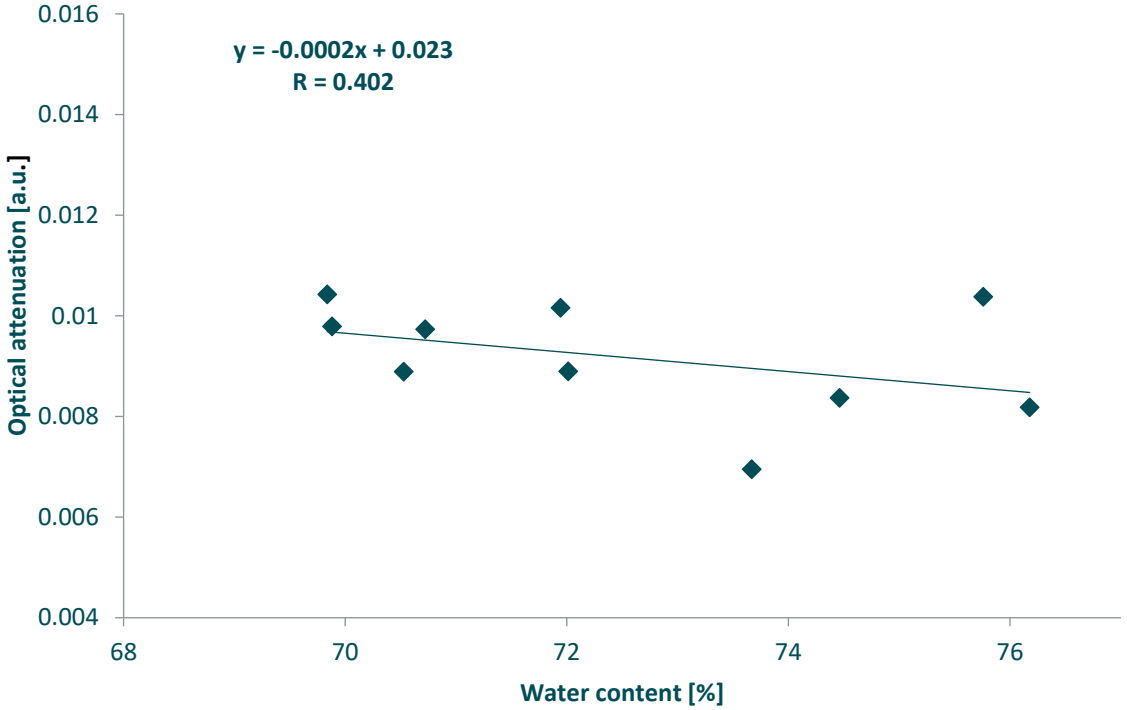
Water Content in the Dermis Correlates with Ageing and Photoageing

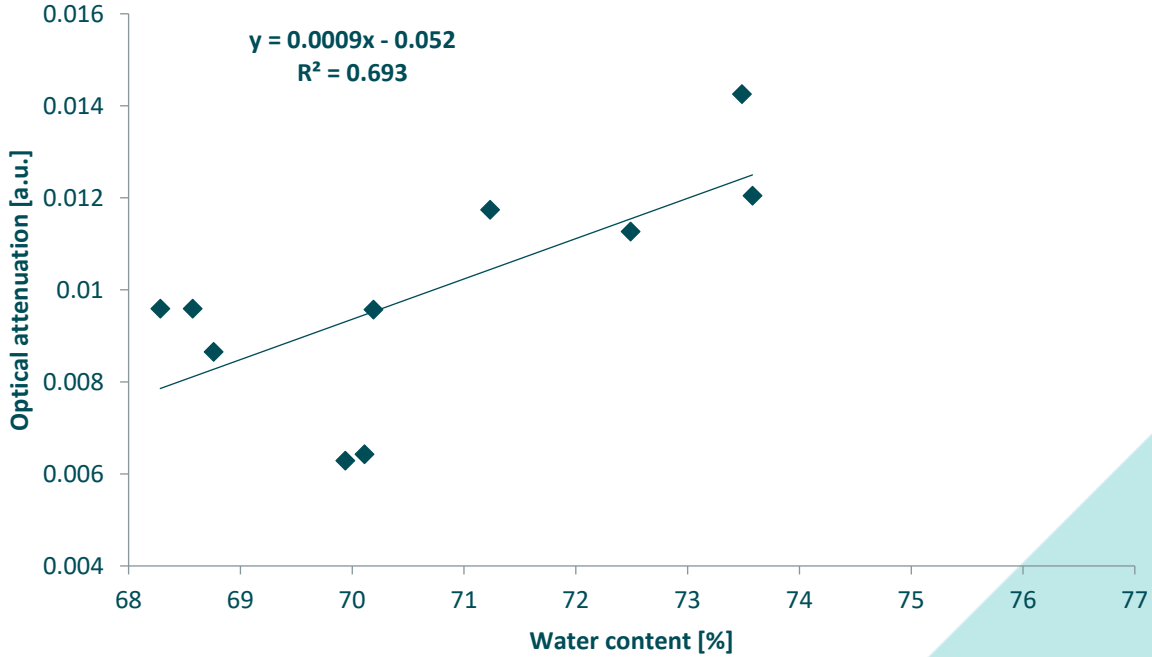


Measurement on the forearm of 23 female subjects at a depth of 130 to 150 µm (upper reticular dermis)

Kourbaj, G., Bielfeldt, S., Seise, M., & Wilhelm, K. P. (2021). Measurement of dermal water content by confocal RAMAN spectroscopy to investigate intrinsic aging and photoaging of human skin in vivo. *Skin Research and Technology*, *27*(3), 404-413.

Does Water Content in the Dermis Correlate with LC_OCT Optical Attenuation?





Measurement on the forearm of 10 female subjects at a depth of 130 to 150 µm (upper reticular dermis)

On sun exposed dorsal skin water seems to replace collagen in ageing upper dermis

Agenda

- Intrinsic and extrinsic aging of the dermis
- Non invasive in-vivo methods to assess ageing of the dermis
  - In vivo assessment of collagen and elastin
  - In vivo assessment of AGE
- Summary

Invasive Measurement Methods of AGEs

Biopsies or Tissue Fluid Required; Non Invasive: CML from SC-Extracts*

Table 1. Detected AGEs in skin*

AGE	Skin compartments involved	Targets of glycation	Methods of detection
CML	Epidermis[18] Aged and diabetic dermis[19-22] Photoaging–actinic elastosis[20,23]	Epidermis (SC -CK10, SS, SG)[18] Collagen[19-21] Vimentin[22] Elastin[20,23]	LC-ESI-TOF-MS, IF, IB[18] SIM/GC-MS[19,21] IHC[20,22,23] ELISA,[23] confocal microscopy[23]
Pentosidin	Aged and diabetic dermis[19,24,25]	Collagen[19,24,25]	Reversed-phase HPLC,[19,24] ELISA,[25] IB[25]
GO	Aged dermis[21]	Collagen[21]	LC/MS[21]
MGO	Aged dermis[21]	Collagen[21]	LC/MS[21]
Glucosepane	Aged dermis[21,26]	Collagen[21,26]	LC/MS[21,26]
Fructoselysine	Aged dermis[21]	Collagen[21]	LC/MS[21]
CEL	Aged dermis[21,27]	Collagen[21,27]	LC/MS[27] SIM/GC-MS[21]
GOLD	Aged dermis[28]	Collagen[28]	LC/MS[28]
MOLD	Aged dermis[28]	Collagen[28]	LC/MS[28]

ELISA, enzyme-linked immunosorbent assay; GO, glyoxal; HPLC, high performance liquid chromatography; IHC, immunohistochemistry; IB, immunoblotting; IF, immunofluorescence; LC-ESI-TOF-MS, liquid chromatography–electrospray ionization time-of-flight mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; MGO, methylglyoxal; SIM/GC-MS, selected ion monitoring gas chromatography-mass spectrometry; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum; all other abbreviations are already explained in the text.

Gkogkolou, P., & Böhm, M. (2012). Advanced glycation end products: Key players in skin aging?. *Dermato-endocrinology*, *4*(3), 259-270.

*Kawabata, K., Yoshikawa, H., Saruwatari, K., Akazawa, Y., Inoue, T., Kuze, T., ... & Sugiyama, Y. (2011). The presence of Nε-(Carboxymethyl) lysine in the human epidermis. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1814(10), 1246-1252.

In Vivo Measurement of AGEs

AGE Reader

- The AGE Reader is a non-invasive monitoring medical device
- UV light is used to excite autofluorescence in human skin tissue.
- Skin autofluorescence is evaluated to roughly quantify the level of AGEs

Variables	AF (a.u.)	CLF (a.u./ µg hyp.)	Pentosidine (pmol/ µg hyp.)	CML (mmol/ mol lysine)	CEL (mmol/ mol lysine)
AF (a.u.)	-	r=0.62 **	r=0.55 **	r=0.55 **	r=0.47 **
CLF (a.u./ µg hyp.)	r=0.62 **	-	r=0.55 **	r=0.50 **	r=0.37 *
Pentosidine (pmol/ µg hyp.)	r=0.55 **	r=0.55 **	-	r=0.46 **	r=0.41 **
CML (mmol/ mol lysine)	r=0.55 **	r=0.50 **	r=0.46 **	-	r=0.65 **
CEL (mmol/ mol lysine)	r=0.47 **	r=0.37 *	r=0.41 **	r=0.65 **	-

Table 1. Correlation between skin autofluorescence, collagen-linked fluorscence and specific skin AGE levels in validation study for diabetic and control population.

Meerwaldt, R., Links, T., Graaff, R., Thorpe, S. R., Baynes, J. W., Hartog, J., ... & Smit, A. (2005). Simple noninvasive measurement of skin autofluorescence. *Annals of the New York Academy of Sciences*, *1043*(1), 290-298.

Summary

- Ageing and photoageing of the dermis can be assessed in vivo and non invasively
  - Collagen degradation can be assessed with confocal microscopy (CRS, LC-OCT)
  - Multiphoton tomography of the upper dermis can be used to quantify elastin
  - Water content of the dermis (Raman measurements) correlates with age dependent depletion of dermal fiber proteins
  - AGE content can roughly be quantified in vivo (AGE reader);
    advanced in vivo methods would be appreciated

- Mainly radiation from the sun is responsible for the premature ageing of protein structures in the dermis