ANTI-AGEING
SKIN CARE
CONFERENCE
VIRTUAL EDITION 3-5 November 2020

Clinical Signs of Photoageing Correlate with the Water Content in the Dermis

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Agenda

- Confocal Raman Spectroscopy (CRS): In vivo chemical skin-analysis
- Aging and photoaging: How is the dermis affected?
- Measurement of photoaging in vivo: A study on 24 subjects
- Conclusions

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Sir Chandrasekhara Venkata Raman: Nobel Prize 1930

for the Discovery of a New Type of Scattered Light

1930

Nature (*London*) **121** 501–502 (1928)

A new type of secondary radiation

If we assume that the X-ray scattering of the 'unmodified' type observed by Prof. Compton corresponds to the normal or average state of the atoms and molecules, while the 'modified' scattering of altered wavelength corresponds to their fluctuations from that state, it would follow that we should expect also in the case of ordinary light two types of scattering, one determined by the normal optical properties of the atoms or molecules, and another representing the effect of their fluctuations from their normal state. It accordingly becomes necessary to test whether this is actually the case. The experiments we have made have confirmed this anticipation, and shown that in every case in which light is scattered by the molecules in dust-free liquids or gases, the diffuse radiation of the ordinary kind, having the same wavelength as the incident beam, is accompanied by a modified scattered radiation of degraded frequency.

The new type of light scattering discovered by us naturally requires very powerful illumination for its observation. In our experiments, a beam of sunlight was converged successively by a telescope objective of 18 cm aperture and 230 cm focal length, and by a second lens of 5 cm focal length. At the focus of the second lens was placed the scattering material, which is either a liquid (carefully purified by repeated distillation *in vacuo*) or its dust-free vapour. To detect the presence of a modified scattered radiation, the method of complementary light-filters was used. A blue-violet filter, when coupled with a yellow-green filter and placed in the incident light, completely extinguished the track of the light through the liquid or vapour. The reappearance of the track when the yellow filter is transferred to a place between it and the observer's eye is proof of the existence of a modified scattered radiation. Spectroscopic confirmation is also available.

Some sixty different common liquids have been examined in this way, and every one of them showed the effect in greater or less degree. That the effect is a true scattering and not a fluorescence is indicated in the first place by its feebleness in comparison with the ordinary scattering, and secondly by its polarisation, which is in many cases quite strong and comparable with the polarisation of the ordinary scattering. The investigation is naturally much more difficult in the case of gases and vapours, owing to the excessive feebleness of the effect. Nevertheless, when the vapour is of sufficient density, for example with ether or amylene, the modified scattering is readily demonstrable.

C V RAMAN
K S KRISHNAN

210 Bowbazaar Street, Calcutta, India

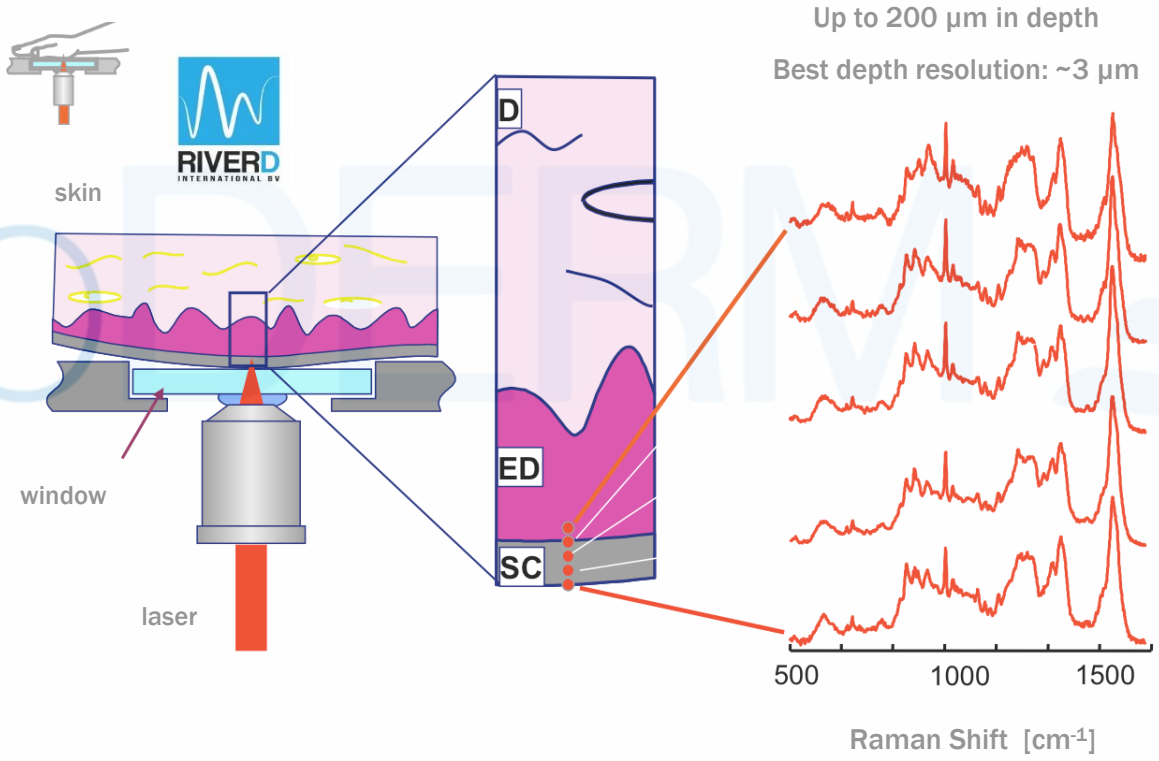
16 February

365

Characteristics of the Raman device (2nd Generation)

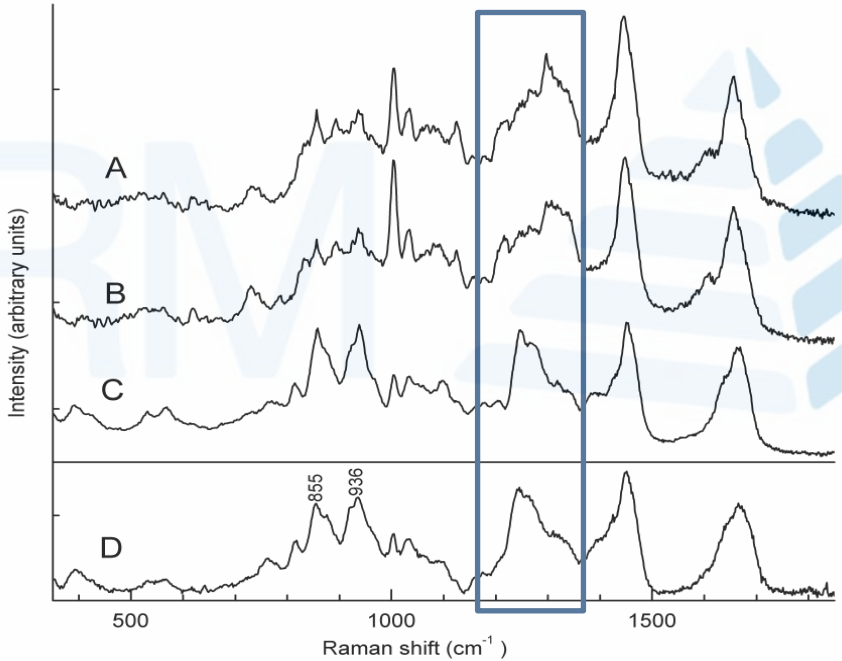
- "gen2-SCA Ultimate" manufactured by RiverD International B. V., Rotterdam, Netherlands
- built-in two lasers (wavelength of 671 nm and 785 nm)
- Movable table in two dimensions
- Adjustable pinholes: 25, 50 and 100 µm
- Resolution: 3, 5 and 10 µm
- Fingerprint spectra (FP): 400 – 1800 cm^{-1}
- High Wave Numbers (HWN): 2500 – 3800 cm^{-1}
- Typical time for a FP spectrum: 5 sec.
- Typical time for a HWN spectrum: 1 sec.

Confocal Raman Spectroscopy (CRS): How it functions



Raman Spectrum of Stratum Corneum (A), Viable Epidermis (B), Dermis (C), and Collagen (D)

- The in vivo Raman spectrum of the dermis shows mainly collagen
- 90 % of the dry tissue of the dermis is collagen
- The spectral difference to keratin and epidermal protein is small but distinct (blue box and peaks at 855 and 936 cm^{-1})[1)]



From Caspers, P. (2003). *In vivo skin characterization by confocal Raman microspectroscopy*

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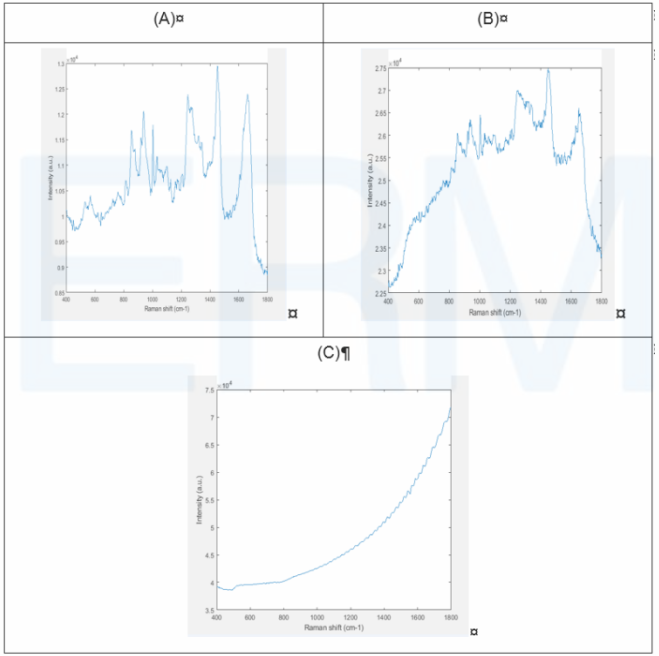
Collagen Spectrum in the Upper Dermis
Fluorescence from Melanin can Disturb the Measurement



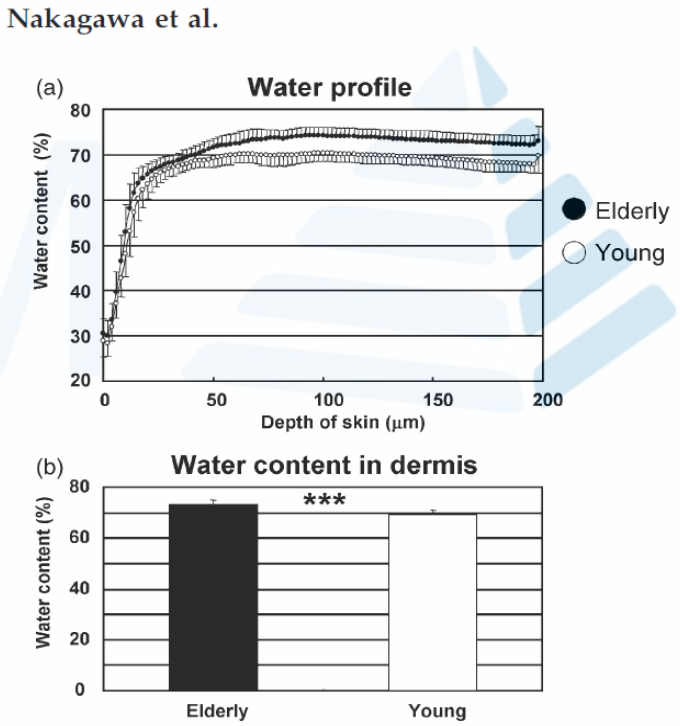
Fluorescence from melanin (B, C) can disturb measurements in the fingerprint region when close to DEJ

The effect is much less dominant for water measurements at high wave numbers

Nakagawa: Water Content in the Dermis is increasing with age

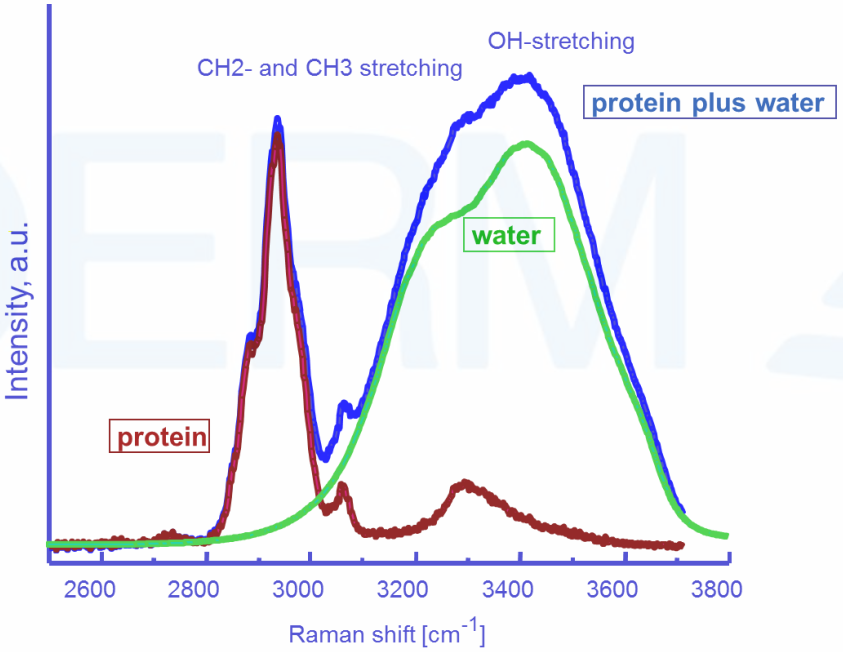
- **Elderly** (60 to 68 years) forearm skin has a **significantly higher water content than young** skin (20 to 24 years)
- Mobile / bulk water was also found to be at higher amounts in the elderly
- This might be due to **reduced amounts of** substrates, **collagen** and glycosamino-glycans in the dermis

Figure 2 from Nakagawa, N., Matsumoto, M., & Sakai, S. (2010). In vivo measurement of the water content in the dermis by confocal Raman spectroscopy. *Skin Research and Technology*, *16*(2), 137-141.

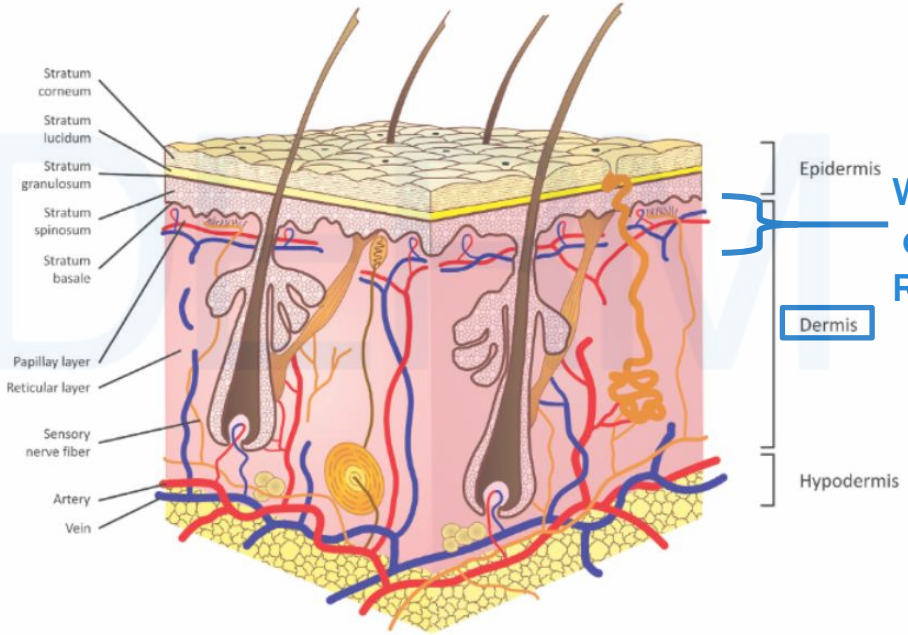


Water Measurement with CRS in the Dermis

High Wave Number Spectra (671 nm Laser)



The Dermis
It is the Main Layer of the Skin, More Than 1 mm Thick

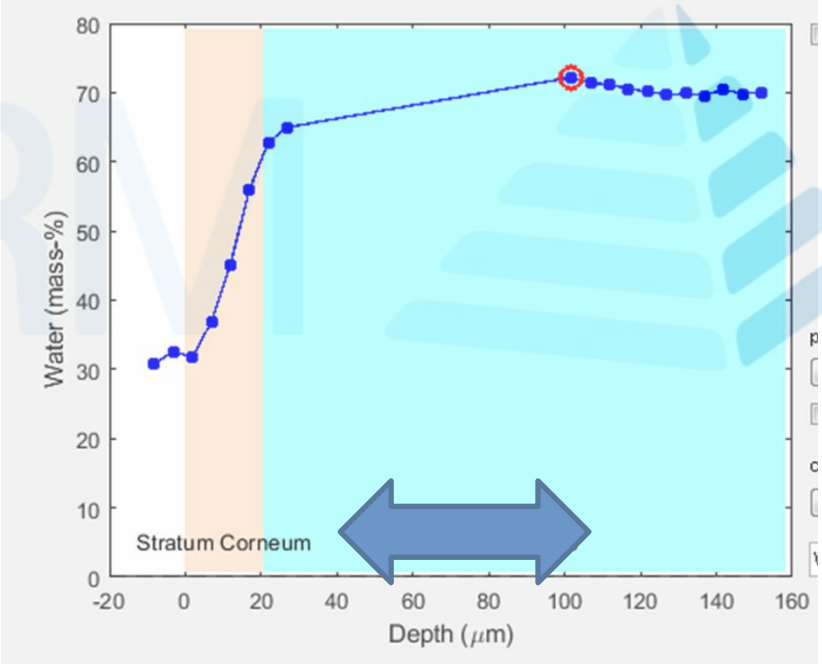


We reach the upper 200 µm of the dermis with Confocal Raman Spectroscopy

From Ventrelli L, Marsilio Strambini L, Barillaro G. Microneedles for transdermal biosensing: current picture and future direction. Adv Healthc Mater 2015; 4(17): 2606-2640

Water Measurement with Confocal Raman Spectroscopy in the Dermis (Pinhole 100 µm, Resolution 10 µm)

- Assessment of 8 – 10 spectra on the forearm in a field of ~ 1 cm²
- Pinhole: 100µm
- Resolution = 10 µm
- Blue double arrow: Range of the epidermal/dermal border
- Automatic surface detection (signal of window/keratin each at 50%)



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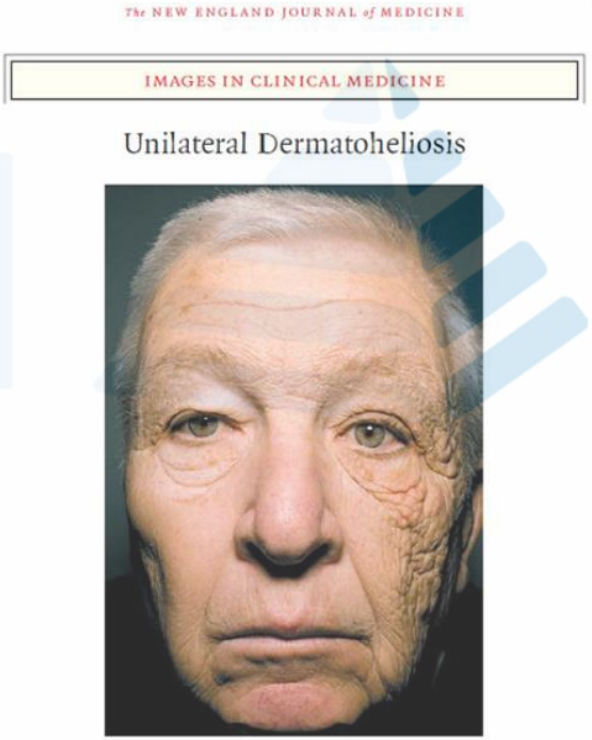
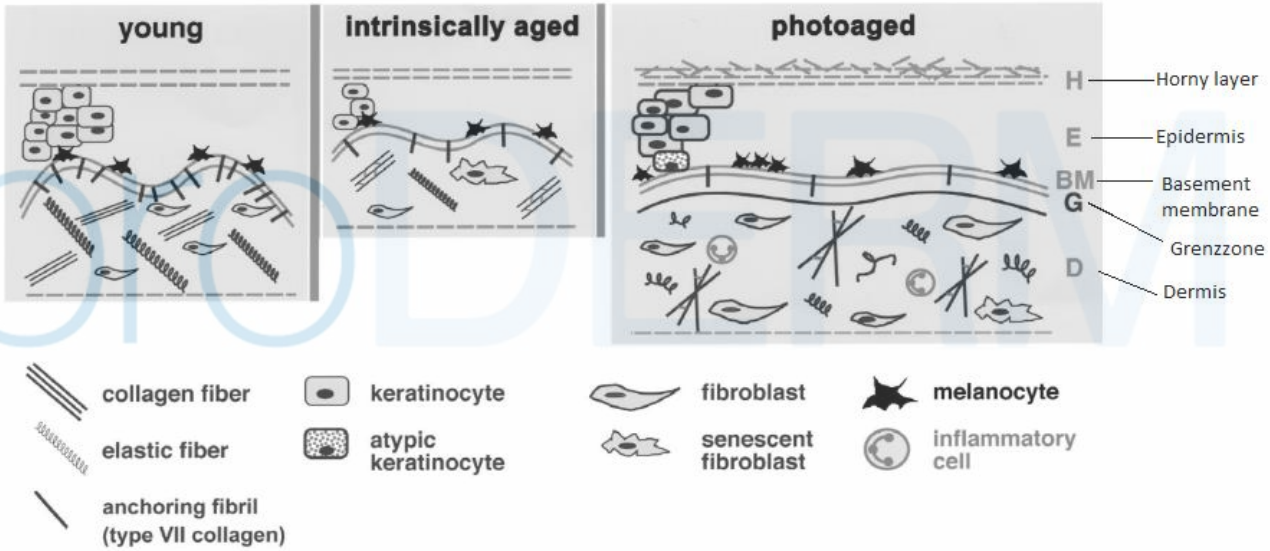
Clinical Signs of Intrinsic Aging and Photoaging of the Dermis

- Intrinsic aging
  - Wrinkled skin
  - Loss of elasticity
  - Less pigmentation
  - Thin and brittle skin

- Photoaging
  - Mottled pigmentation
  - Depigmented spots
  - Rough wrinkles in the sun exposed areas
  - Increased loss of skin elasticity in the sun exposed areas



Structural Signs of Aging and Photoaging



- **Intrinsic aging:** Senescent fibroblast => depletion of collagen and elastin
- **Photoaging:** Damage of anchoring fibrils, **Bulk water increased 30%,** Deposition of damaged elastin

From Wlaschek M, Tantcheva-Poór I, Naderi L, Ma W, Schneider L, Razi-Wolf Z, Schüller J, Scharffetter-Kochanek K. Solar UV irradiation and dermal photoaging. J Photochem Photobiol B 2001; 63(1-3): 41-51.

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Study to Assess Water Content in the Dermis of Subjects with Different Degrees of Photoaged Skin

- It is known that:
  - Older people have more water in the dermis than younger
  - Lost collagen is replaced by water
  - Photoaged dermis contains increased amounts of bulk water

- Our hypothesis was:
  - In photoaged dermis increased water contents additionally to the intrinsic aging effect should be found

- In vivo Raman water measurements in the dermis was used to clear the question

Study Design

- 24 subjects, female, 18 to 69 years old
- 50% had no photoaging (scores 0 to 2)
- 50% had mild to moderate photoaging (scores 4 to 6)

- Water measurement with confocal Raman
  - Dorsal forearm (sun exposed, photoaged)
  - Volar forearm (photo protected control area)

- Measurement of water content of the dermis
  - Depth of 100 to 150 µm
  - Average water content at depth of 130 to 150 µm assessed

- Control, if dermis was reached by assessing a fingerprint spectrum of collagen at a depth of 100 µm

Photoaging Score from McKenzie for subject inclusion

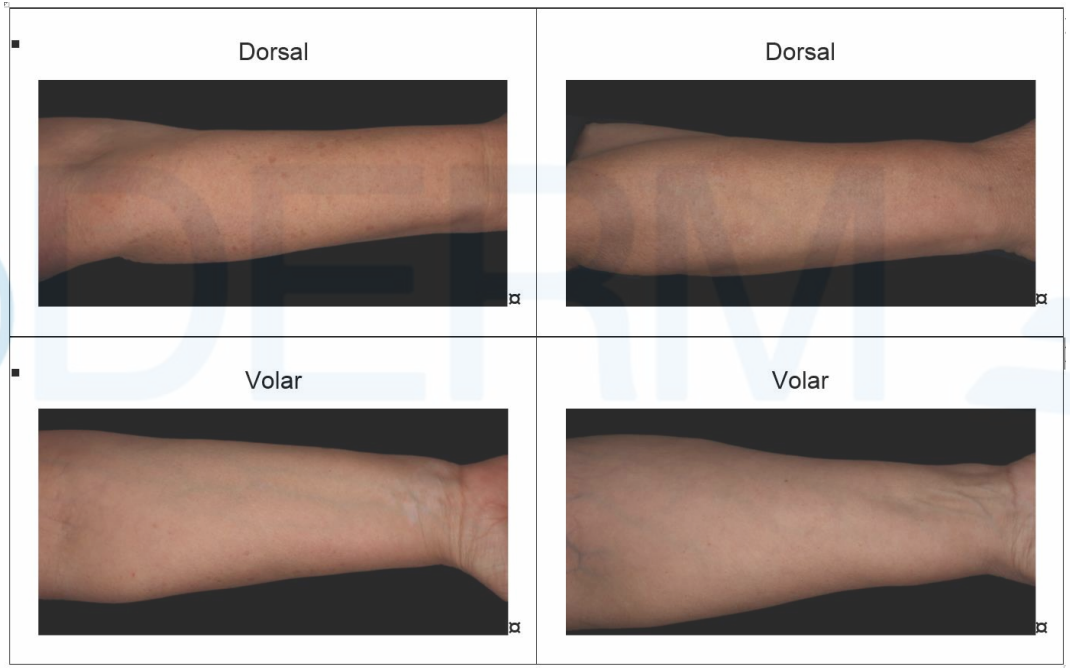
No photoaging

Excluded

Excluded

McKenzie N, Saboda K, Duckett L, Goldman R, Hu C, Curiel-Lewandrowski C. Development of a photographic scale for consistency and guidance in dermatologic assessment of forearm sun damage. Arch Dermatol 2011; 147(1): 31-36.

High Resolution Photography of Dorsal and Volar Forearms proDERM HiRIS System with Canon 5D Mark III Camera



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High Resolution Photography of Dorsal and Volar Forearms Subjects with Moderate Photoaging; Center of the Dorsal Forearm

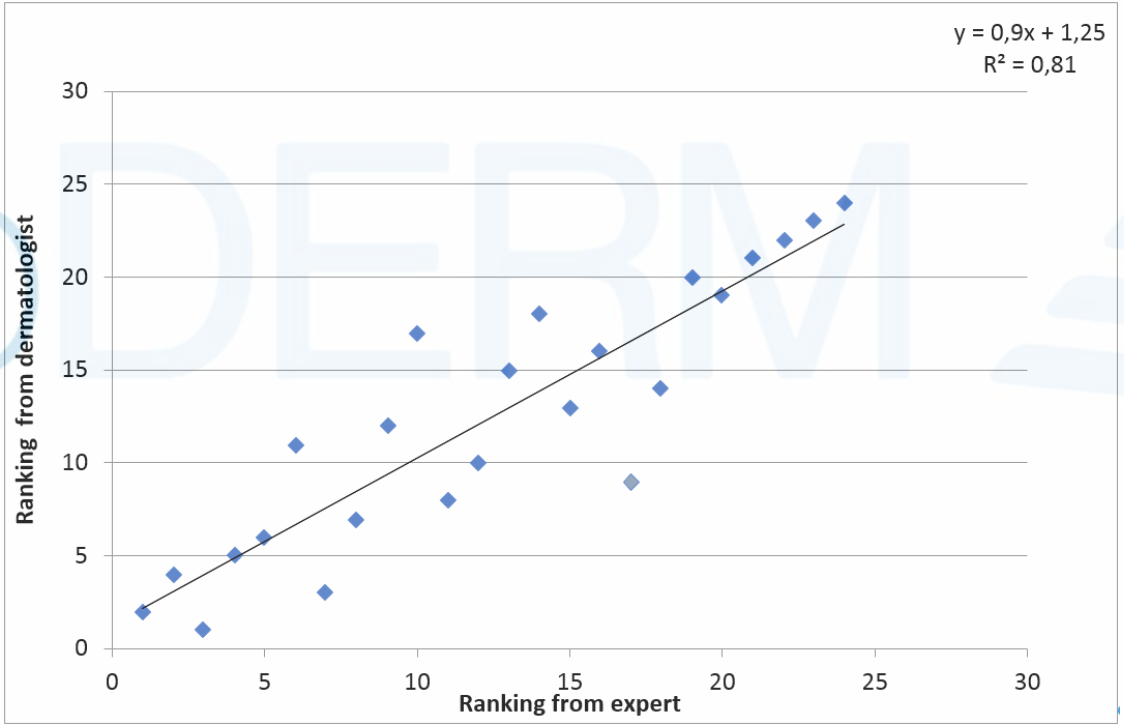
Magnification of original photographs = 4.5

Results: High Resolution Photography of Dorsal Forearms

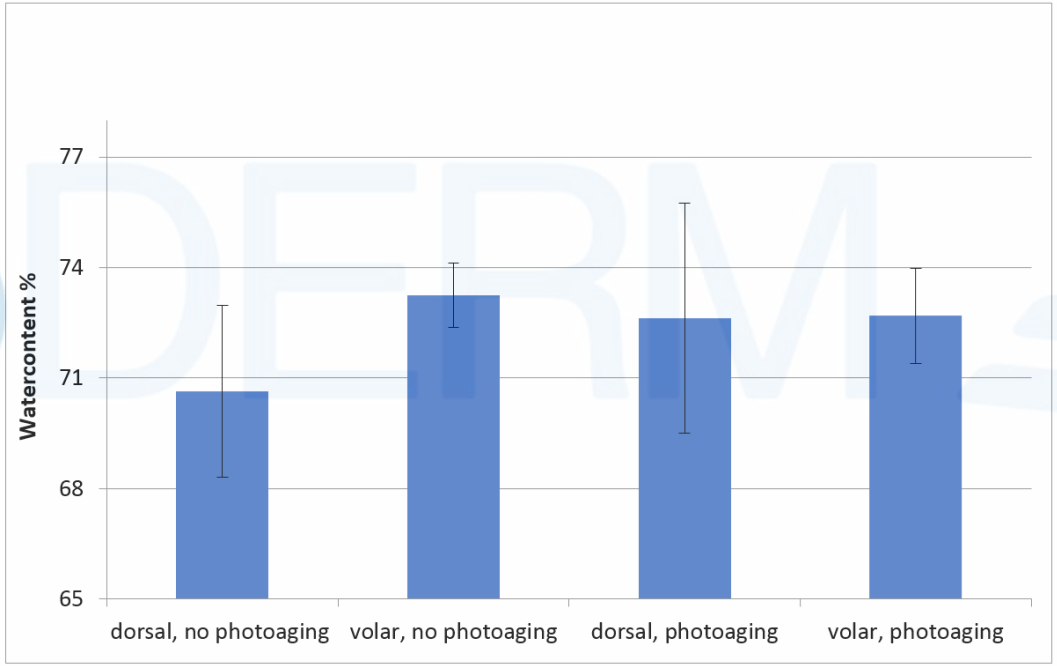
McKenzie Score versus Image Ranking by Two Experts

Subject no.	Age	Photoaging Score according to McKenzie [54] 0 to 2 = no photoaging 4 to 9 = photoaging	Ranking of photoaging from expert 0 Minimum photoaging 24 maximum photoaging	Ranking of photoaging from dermatologist 0 Minimum photoaging 24 maximum photoaging
1	55	4	23	23
2	44	1	7	3
3	61	5	24	24
4	20	0	3	1
5	40	2	12	10
6	29	4	16	16
7	24	1	1	2
8	37	4	14	18
9	48	1	9	12
10	26	4	2	4
11	22	4	6	11
12	18	4	17	9
13	47	2	20	19
14	44	1	5	6
15	54	4	21	21
16	47	2	15	13
17	22	1	10	17
18	51	2	11	8
19	38	4	13	15
20	21	0	4	5
21	45	4	19	20
22	62	6	22	22
23	64	2	18	14
24	21	4	8	7

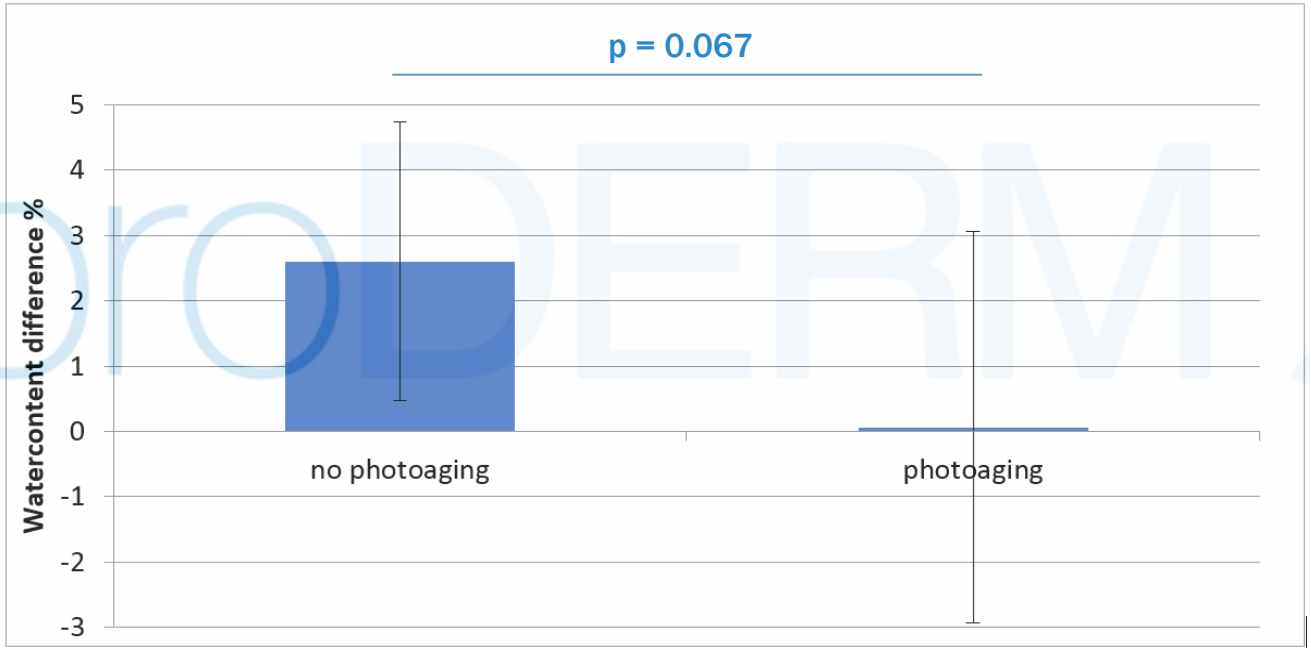
Ranking of Photoaging by Expert and Dermatologist
The Correlation was Excellent: Further Evaluation was Done with the Expert-Data



Mean Water Content in the Dermis at 130 to 150 µm
n = 11 to 12, Means and 95 % Confidence Limits



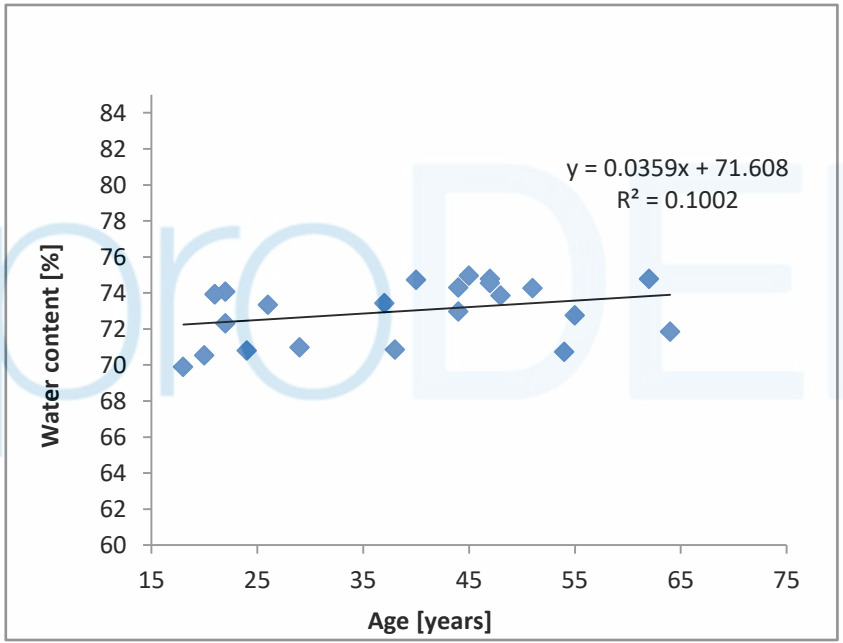
Difference Inner Minus Outer Arm (n = 11)
Mean Water Content in the Dermis 130 to 150 µm;
Means and 95 % Confidence Limits

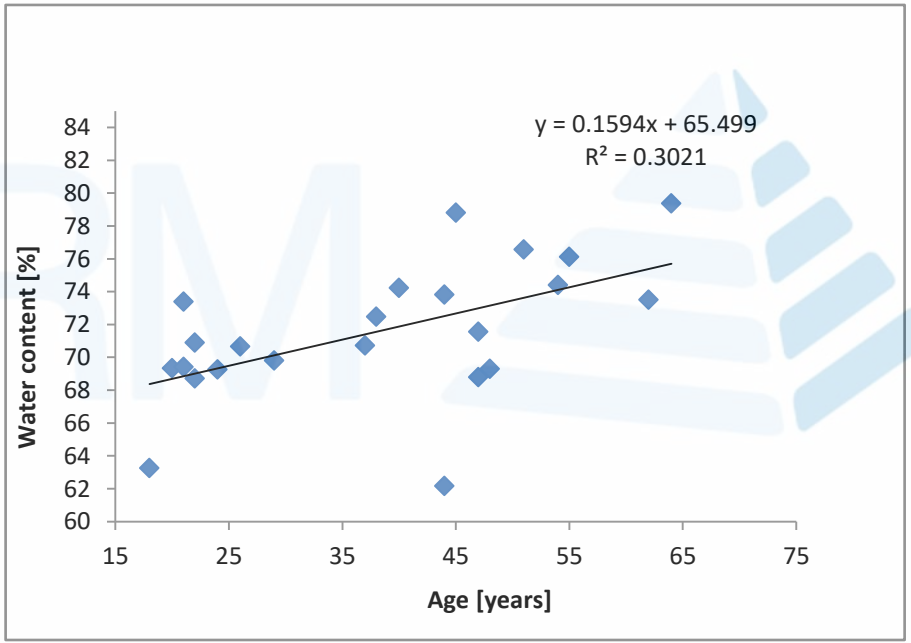


Big surprise: The dorsal forearm contained less water / meaning more collagen than the volar forearm in case it is not photoaged (p = 0.021)!

Due to mild to moderate photoaging the dorsal water content increases and reaches the content of the volar forearm

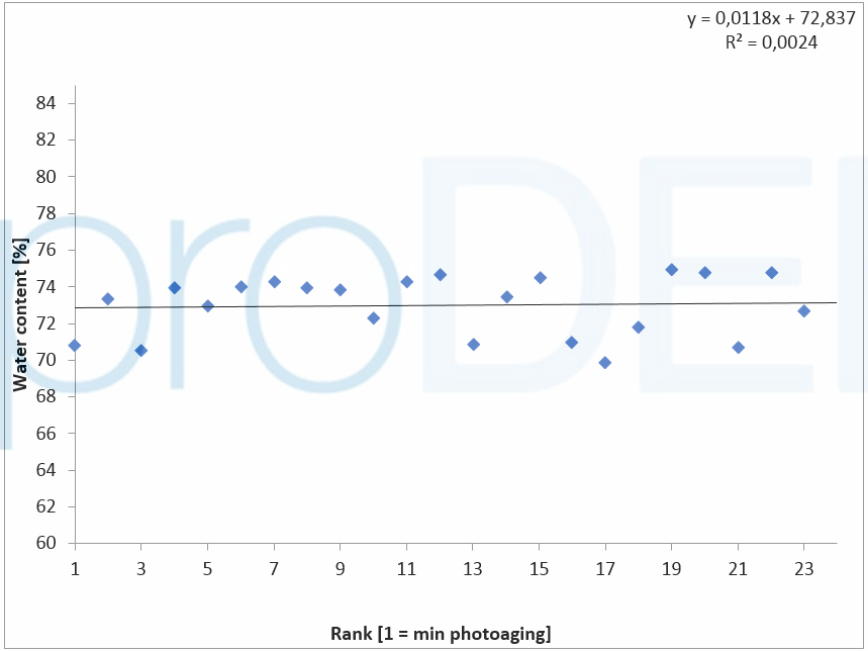
Correlation of Water Content, Volar (Left) and Dorsal (Right) with Age, n = 23

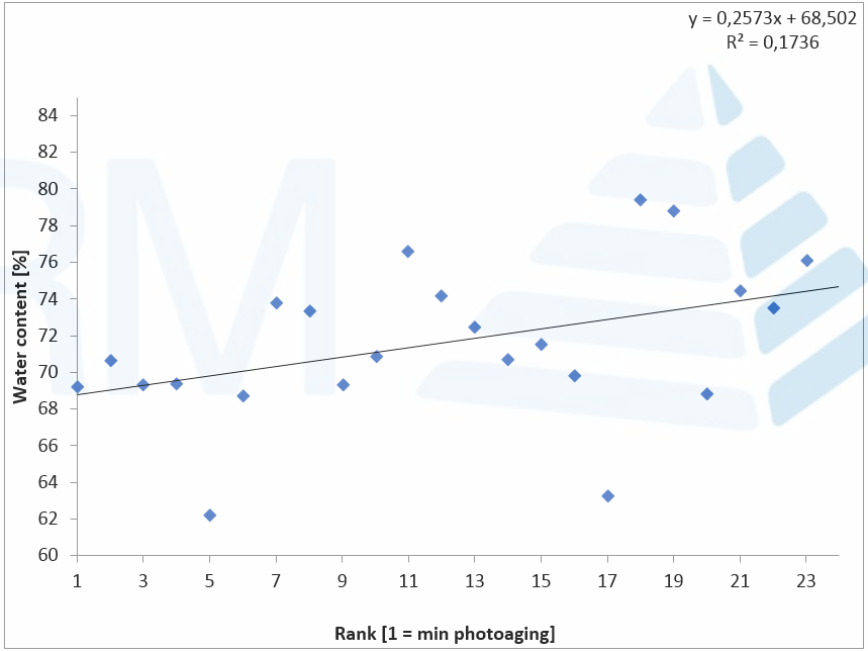




In cases of to mild to moderate photoaging there is a distinct correlation of water content with age.
It is clearly higher compared to photoprotected volar skin (Pearson's r = 0.550 versus r = 0.316)

Correlation of Water Content, Volar (Left) and Dorsal (Right) with Dorsal Photoaging ranks, n = 23



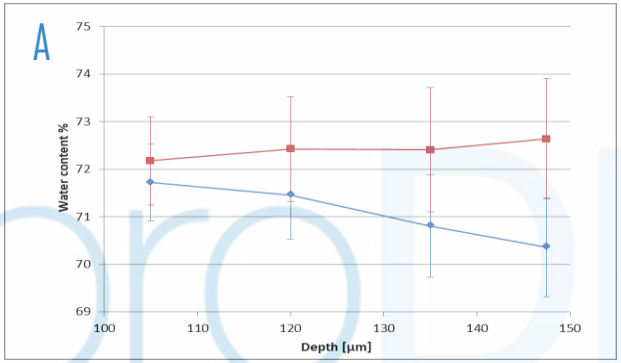


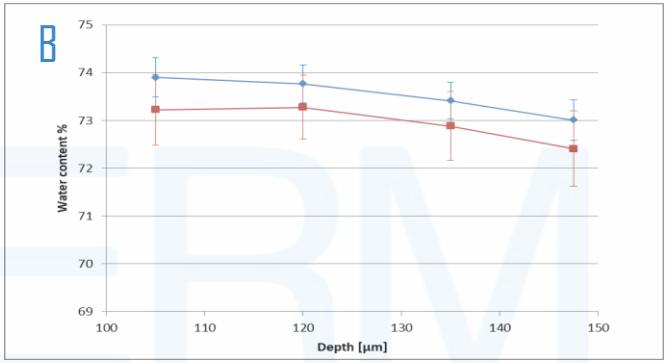
In case of dorsal photoexposed skin a correlation of water content in the dermis with photoaging scores was observed, but not on volar skin (Pearson's r = 0.417 versus r = 0.045)

Mean Water Content in the Dermis from 100 µm to 150 µm

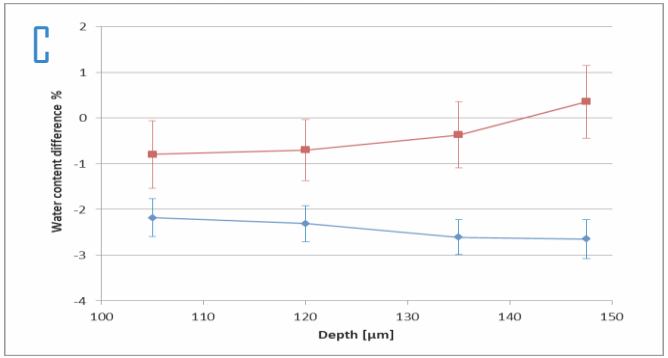
n = 23, Means and Standard Error (SEM)

(A) outer arm (n =12), (B) inner arm (n = 11), (C) inner – outer arm (n = 11)





Why is the effect of photoaging increasing and not decreasing with depth? Deposition of dysfunctional Elastin?



Blue color = no photoaging

Red color = photoaged skin

Conclusions

- Water content in the dermis increases with age but even more with photoaging
- Even in a small panel with mild to moderate photoaging a clear correlation to water content in the dermis was observed
- High resolution photography and image ranking of photoaging is superior to the published photo score of dorsal forearm skin
- The water content of dorsal non photoaged skin is naturally higher compared volar forearm skin The dorsal site seems to be more rich in collagen!
- For topical and orally applied products studies to investigate „increase the collagen content in the skin“ could be performed with this method