In vivo skin analysis by Raman spectroscopy

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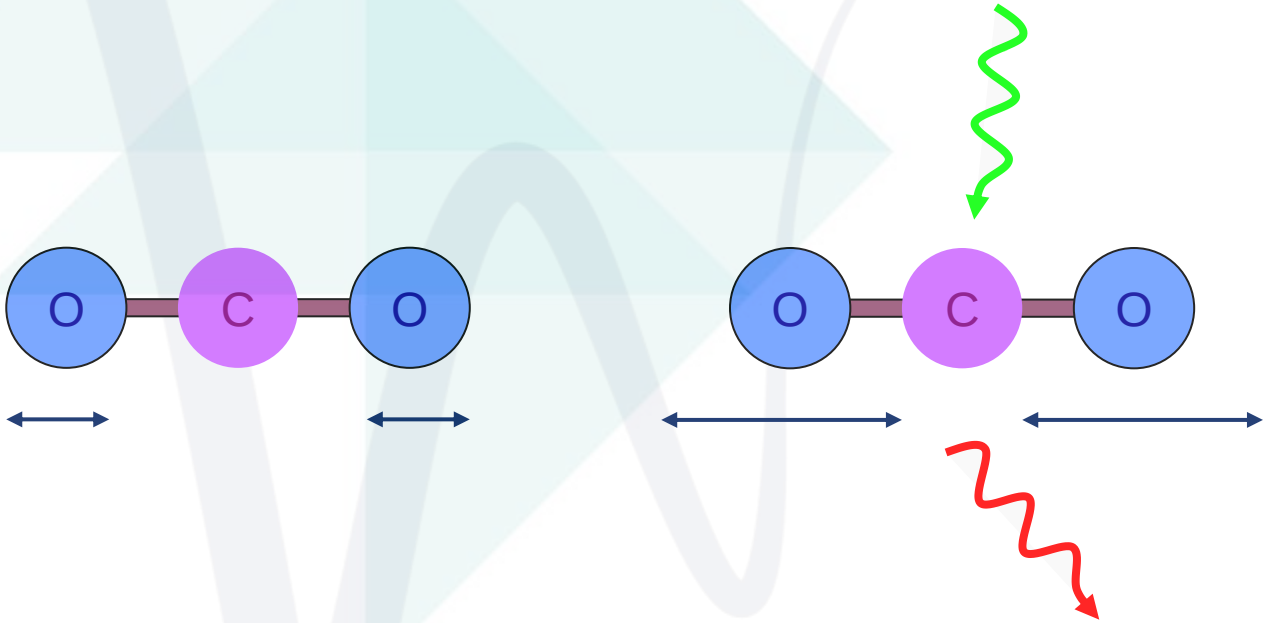
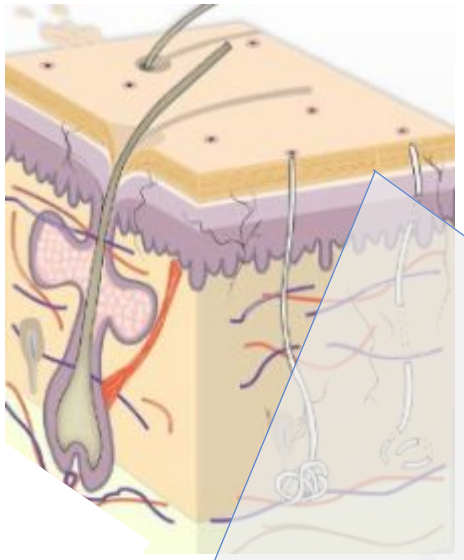
Contents

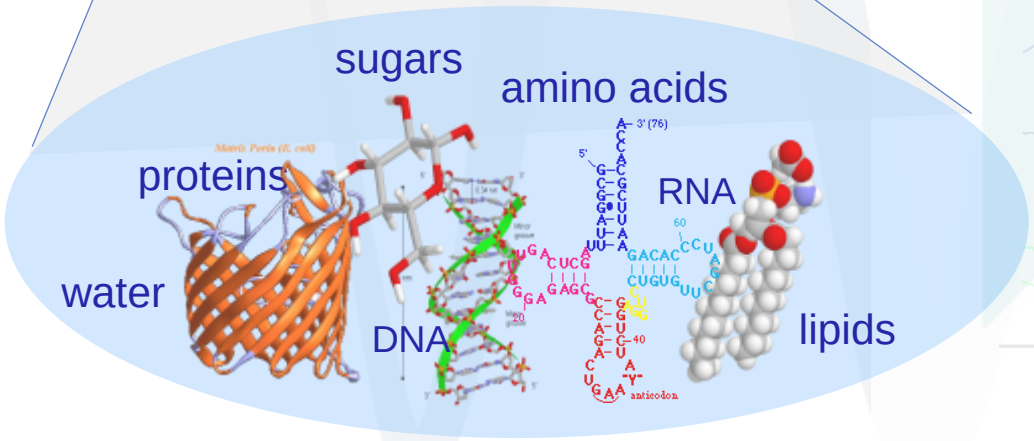
- *in vivo* Raman spectroscopic skin analysis
- Non-invasive, quantitative analysis of topical product penetration

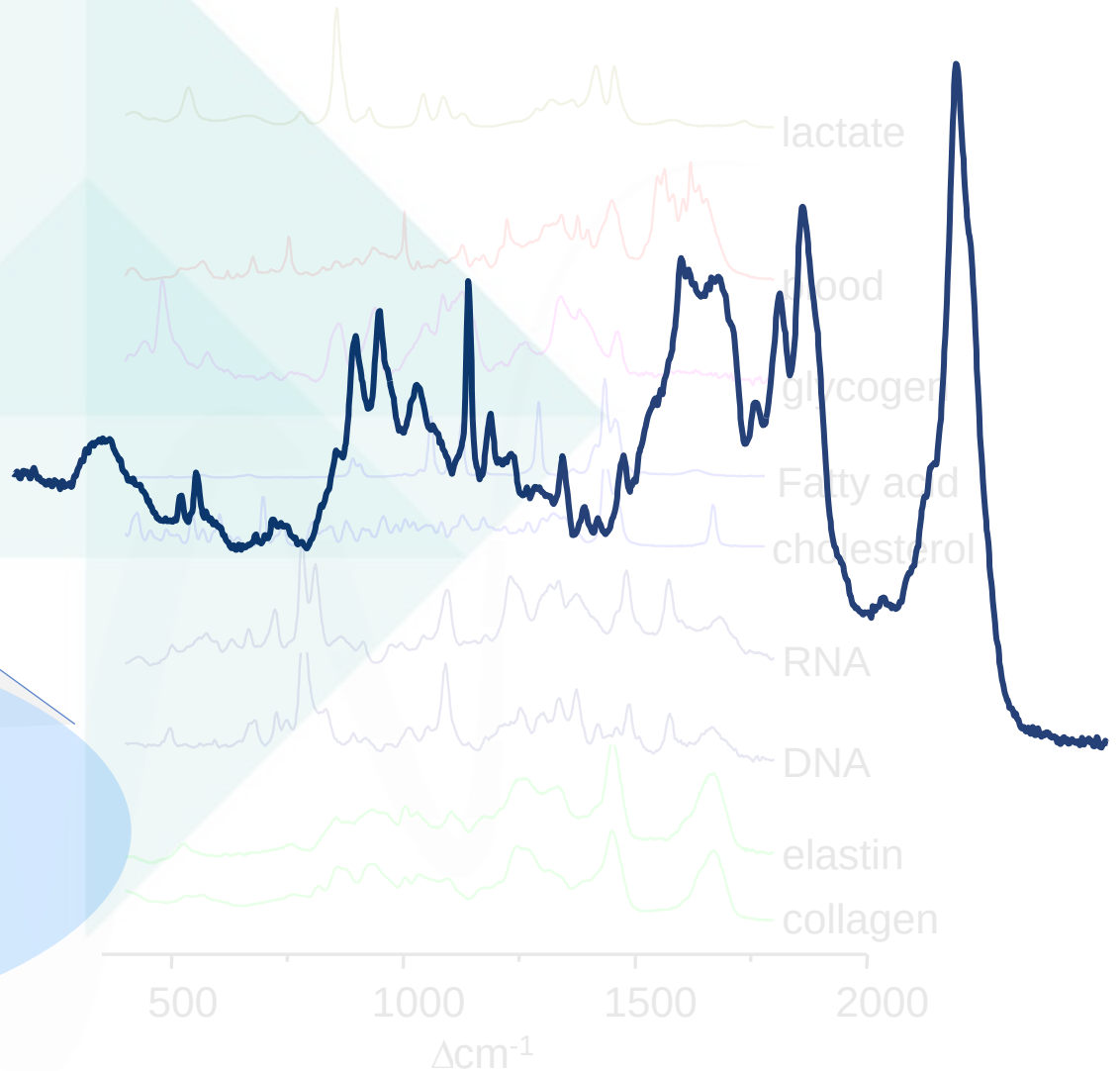
Raman spectroscopy



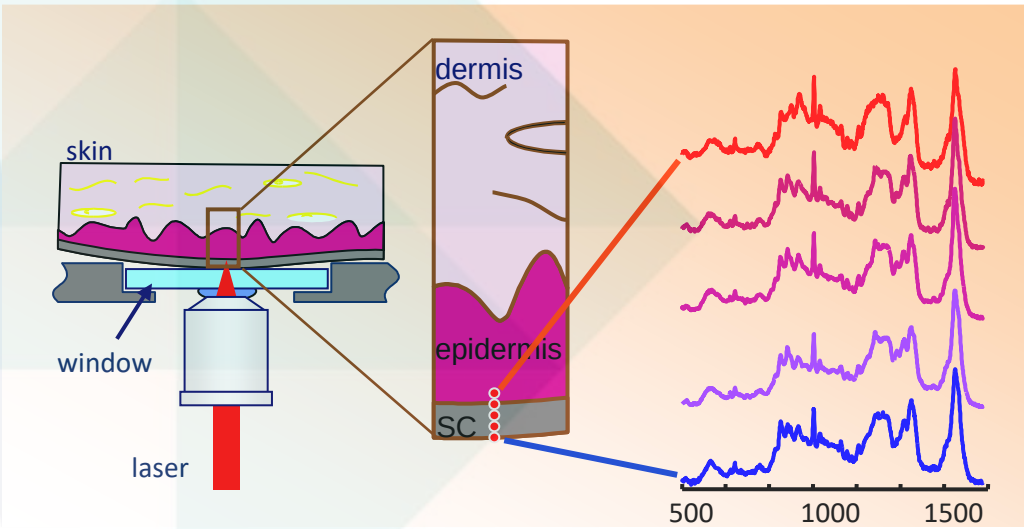
Raman spectroscopy

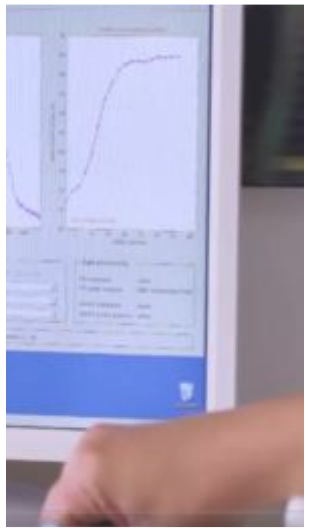






in vivo molecular skin analysis by Raman spectroscopy

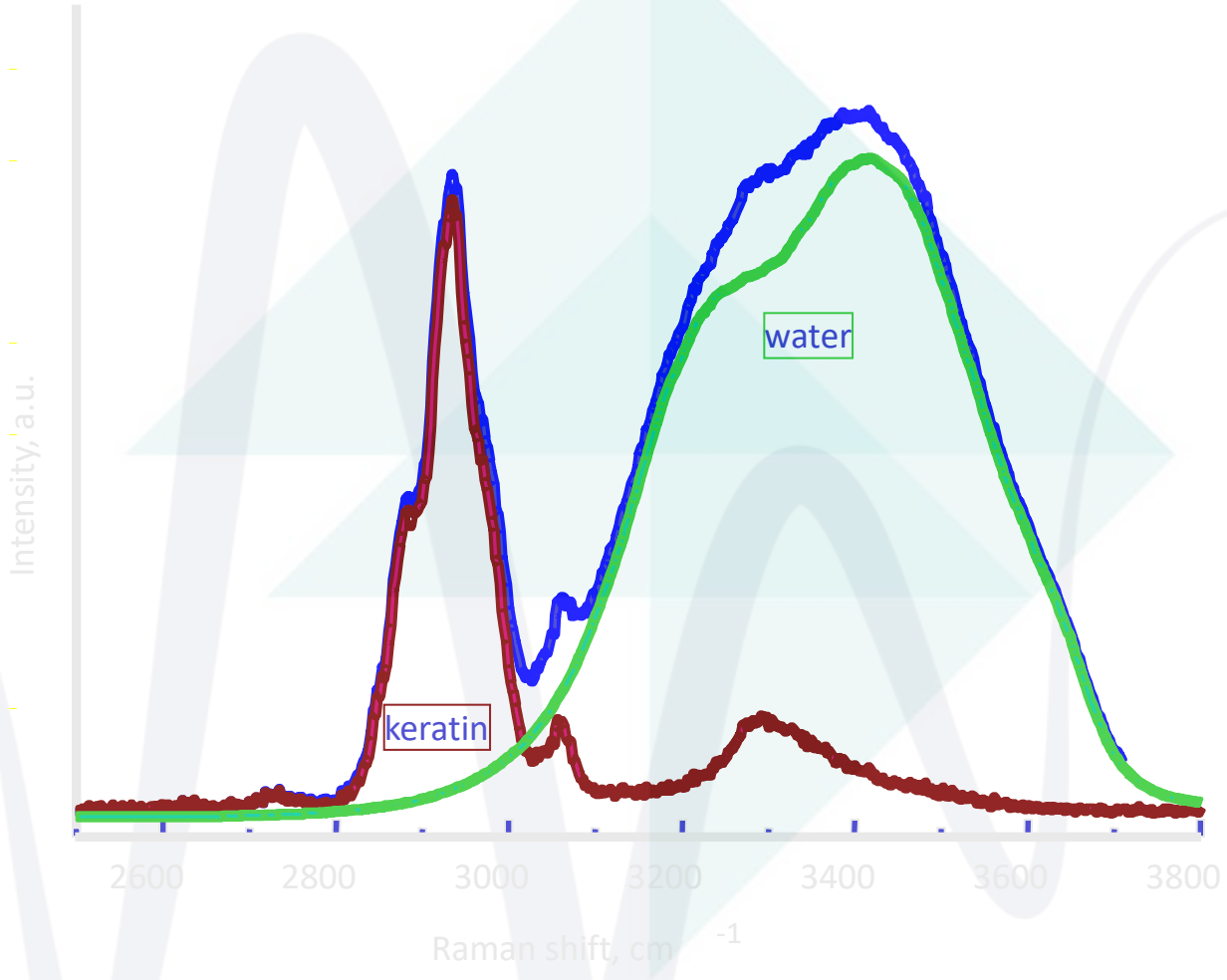




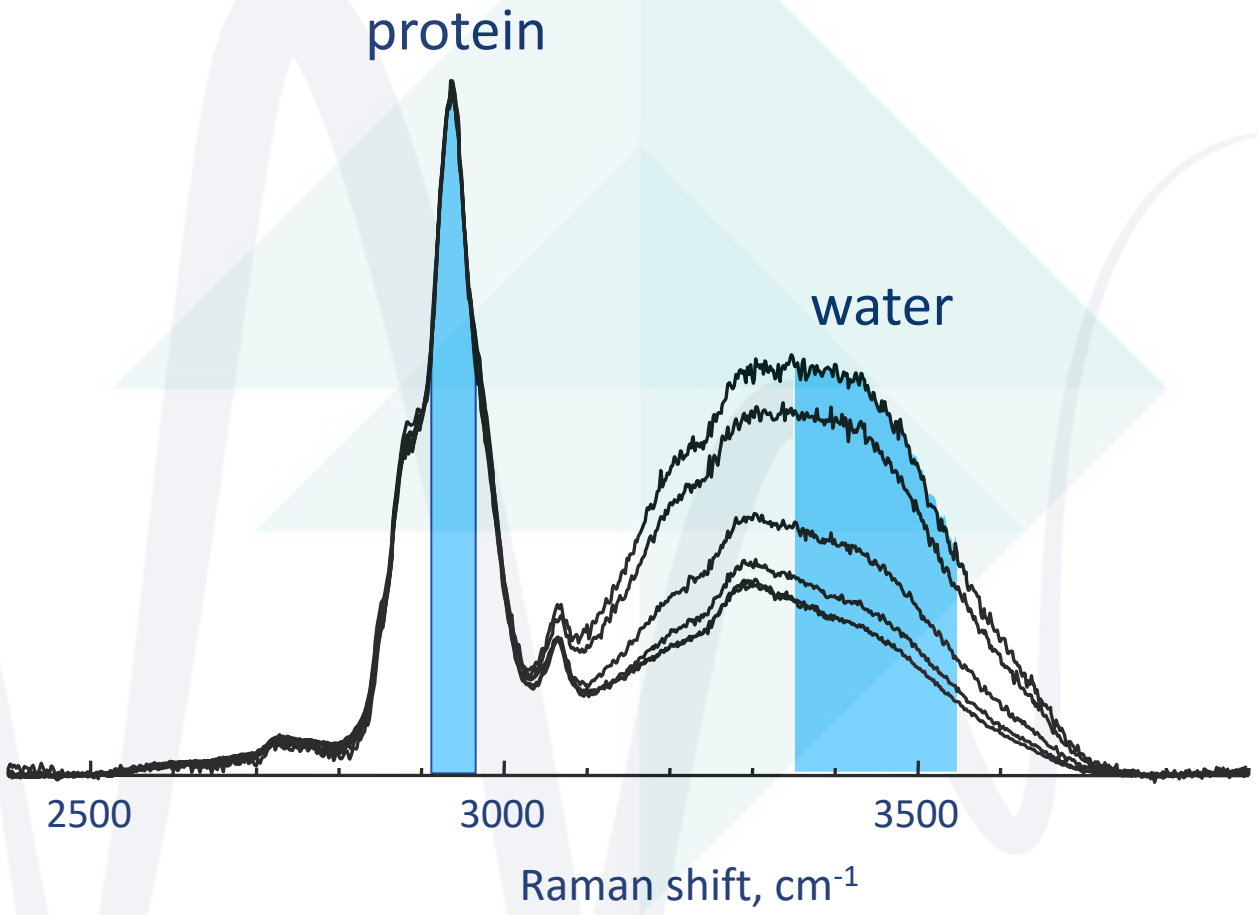
gen2-SCA: spatially resolved analysis of the molecular composition of the skin

SkinTools 3: data analysis

skin hydration: water concentration profile



skin hydration: water concentration profile



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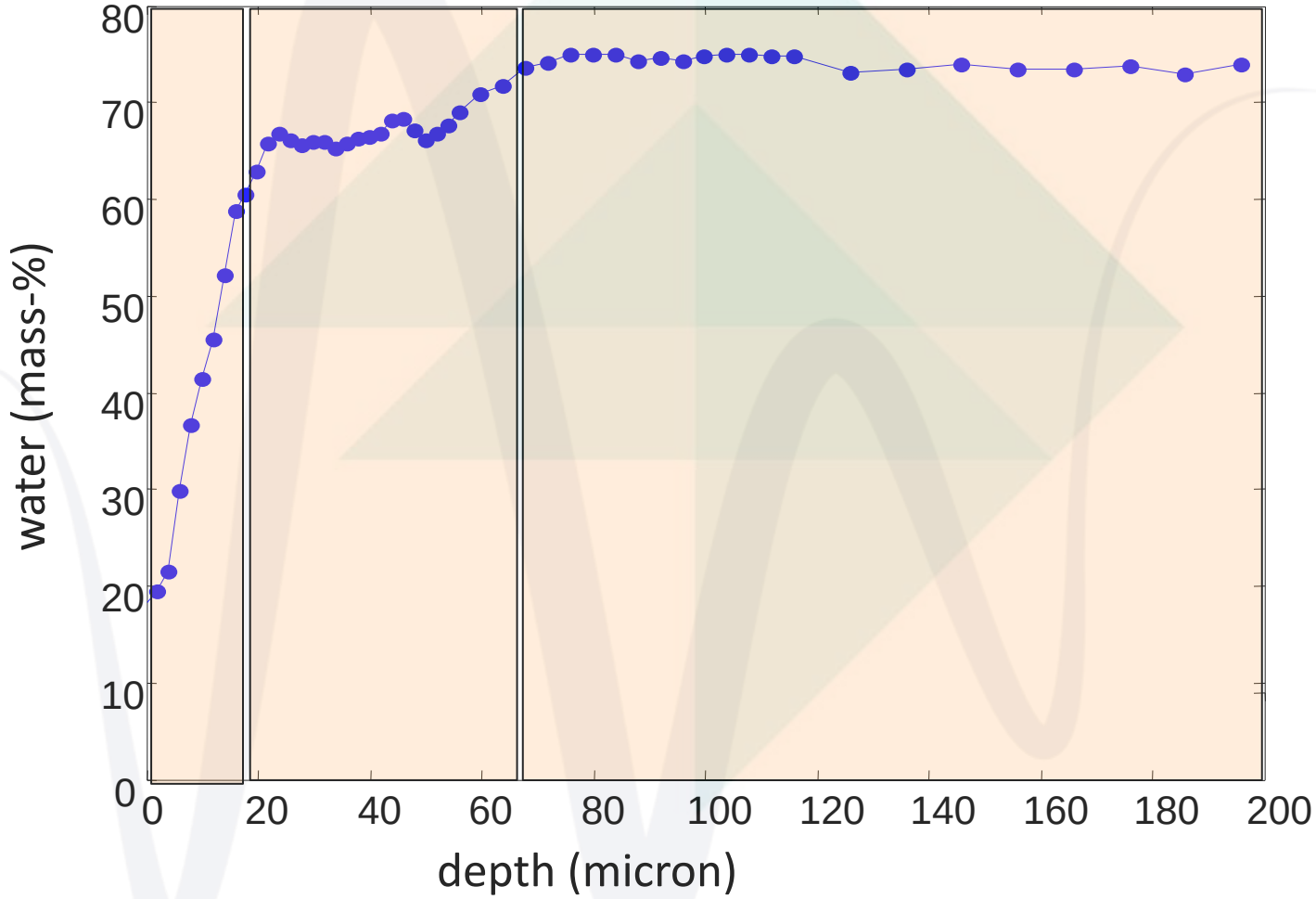
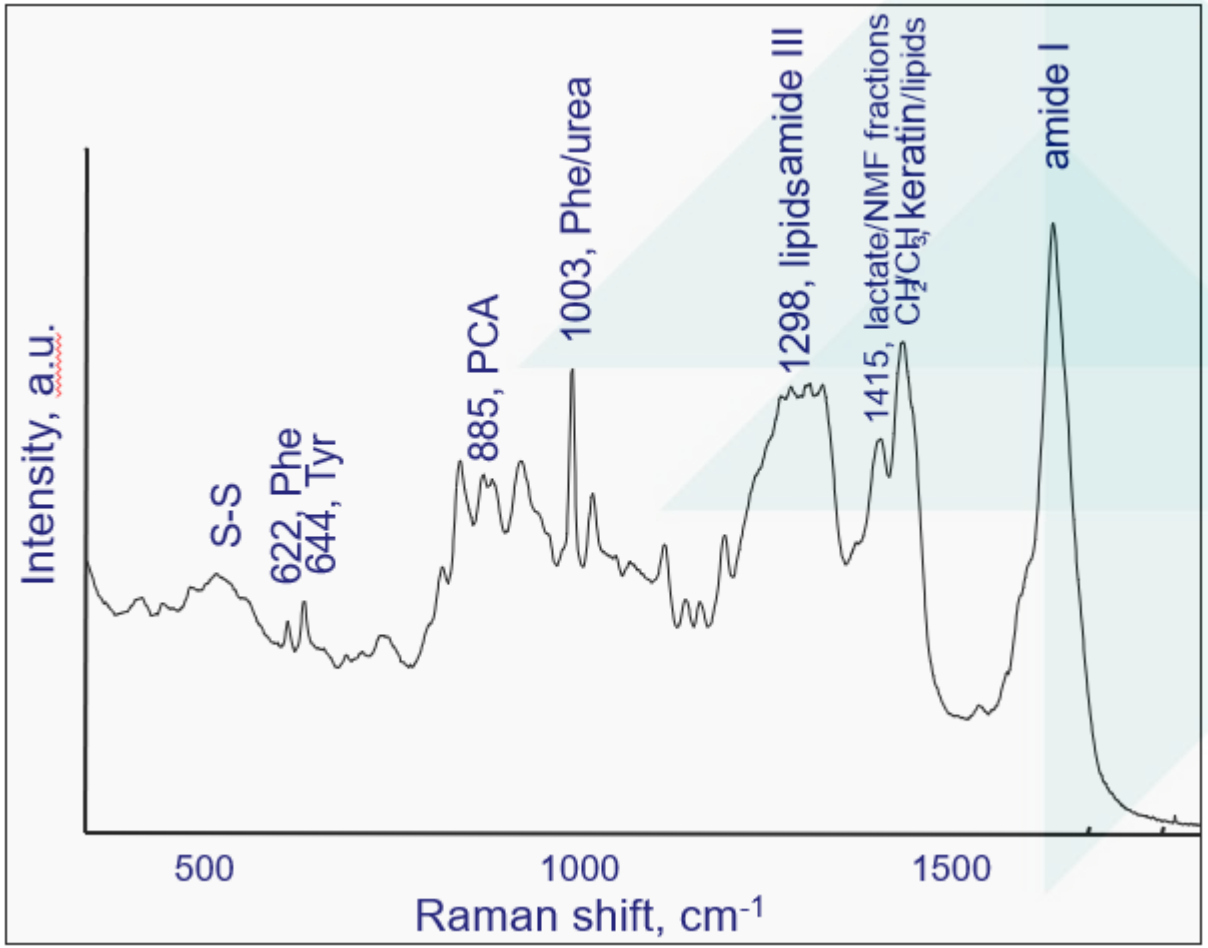
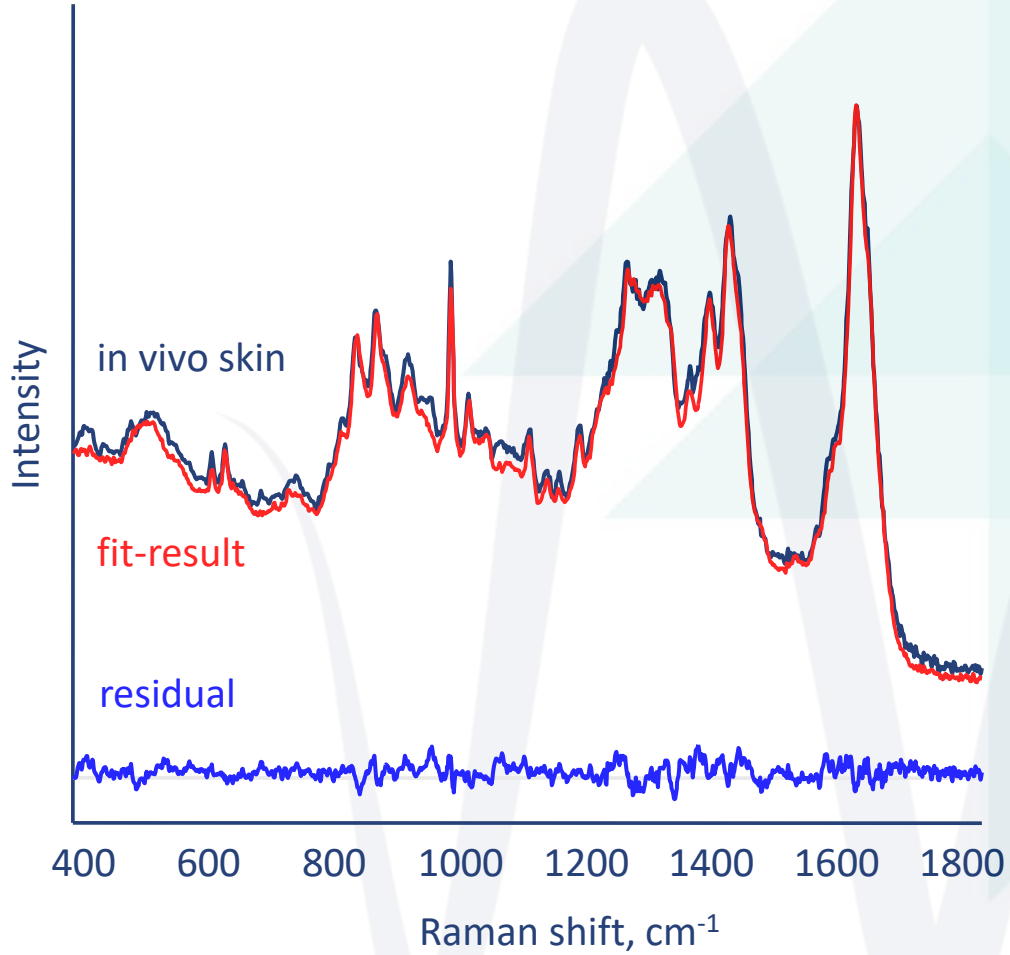


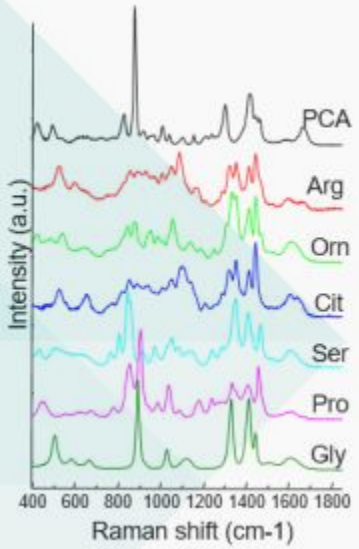
Raman spectroscopy & *in vivo* skin analysis

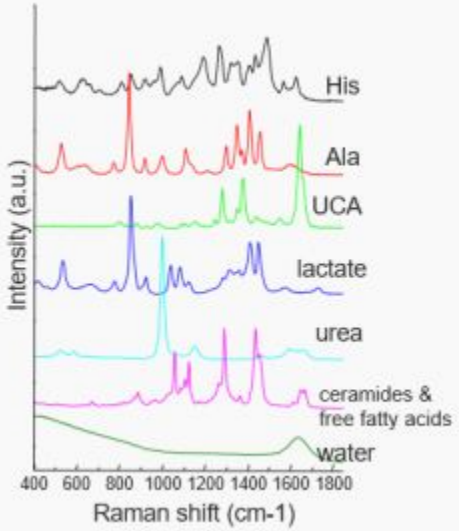


- Raman spectrum of stratum corneum
- Signal contributions of:
  - Proteins
  - amino acids
  - sweat constituents
  - lipids
  - water

Raman spectroscopy & *in vivo* skin analysis

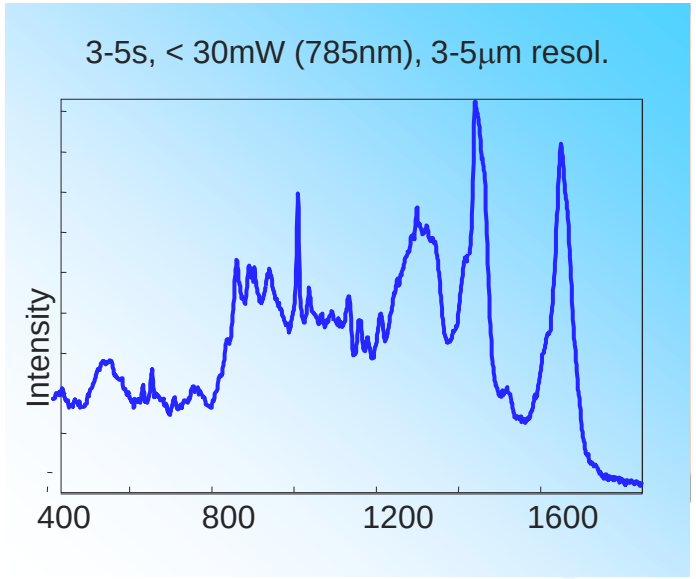


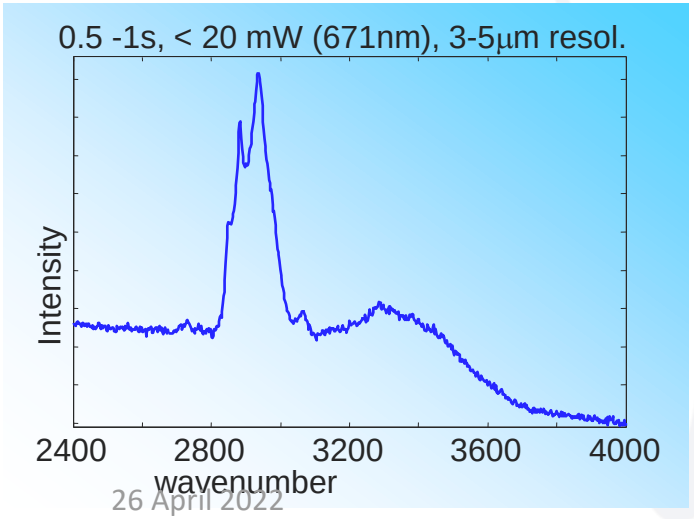




gen2-SCA

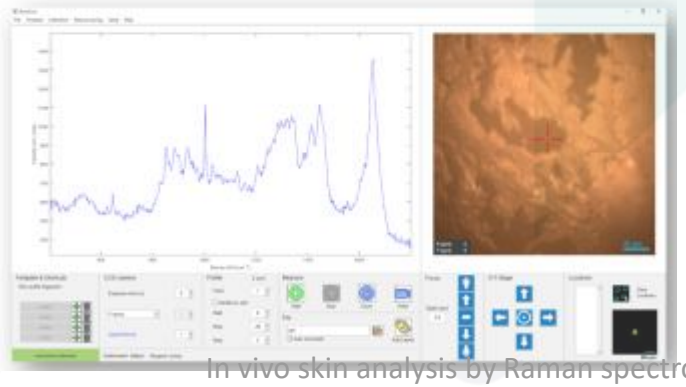
in vivo skin analysis by confocal Raman spectroscopy





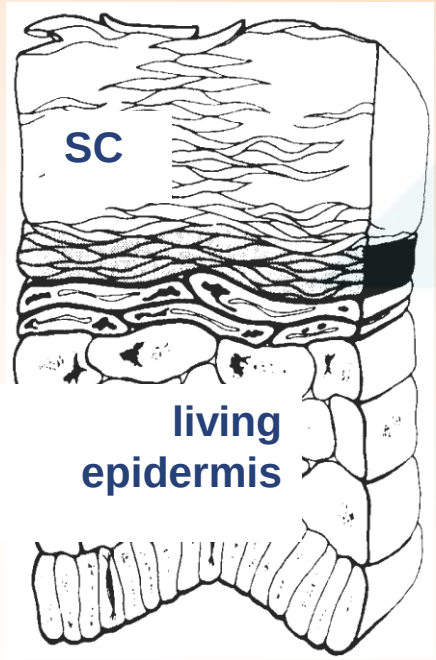
RiverICon 4.3

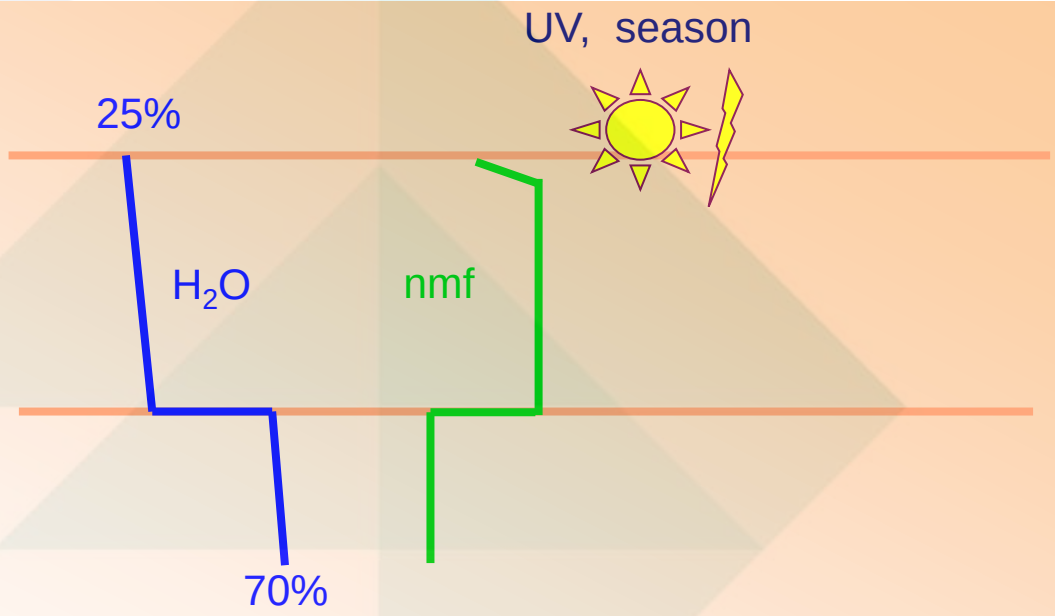
Instrument control software



- **Raman-measurement templates** with user selected:
  - measurement range
  - step-size
  - signal collection time
- **Selection of measurement locations** in skin surface image
- **Autofocusing**
- **Automated profile measurements** at all selected measurement locations

in vivo molecular skin analysis by Raman spectroscopy

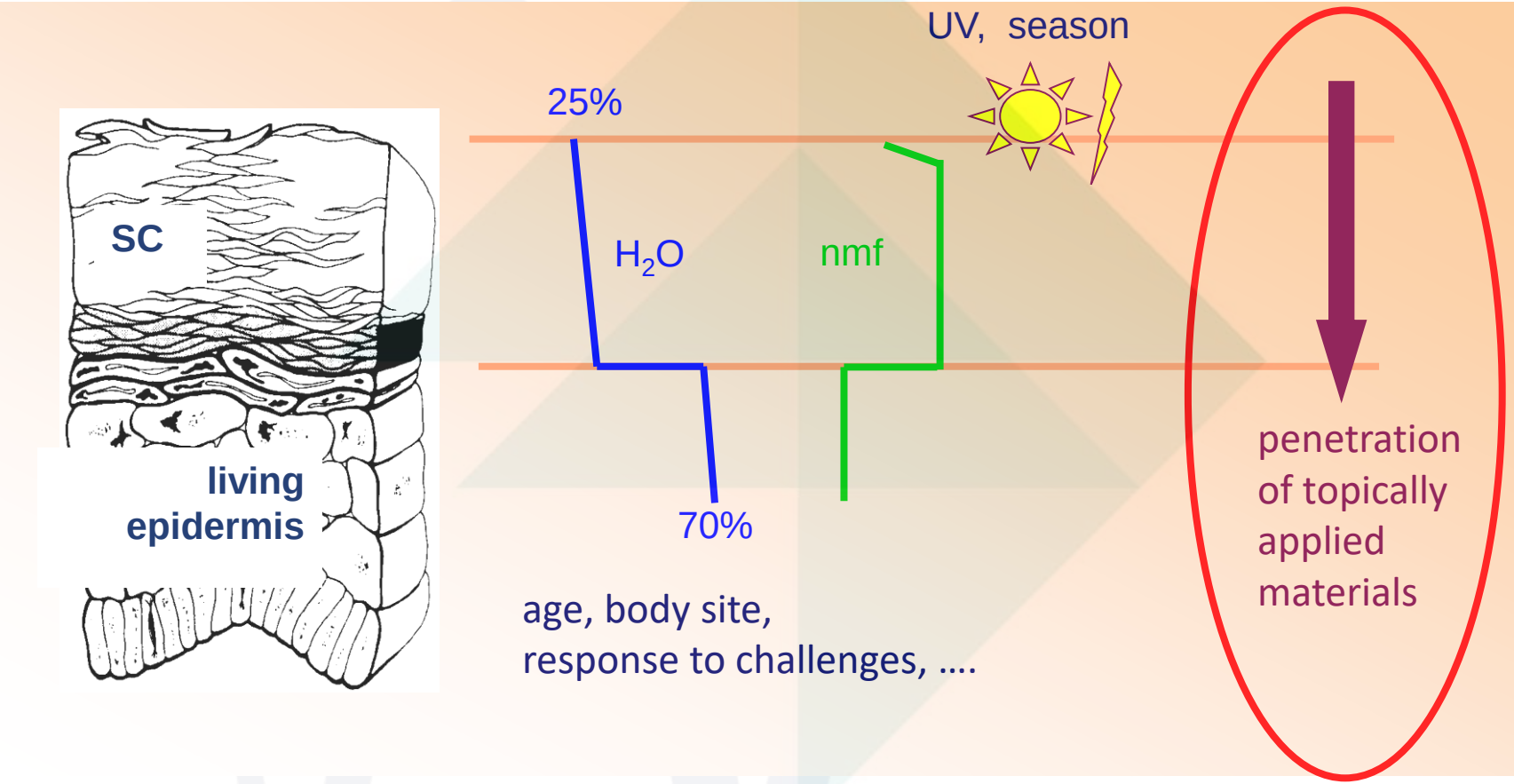




age, body site,
response to challenges, ….

> 150 papers in peer-reviewed scientific literature

in vivo molecular skin analysis by Raman spectroscopy



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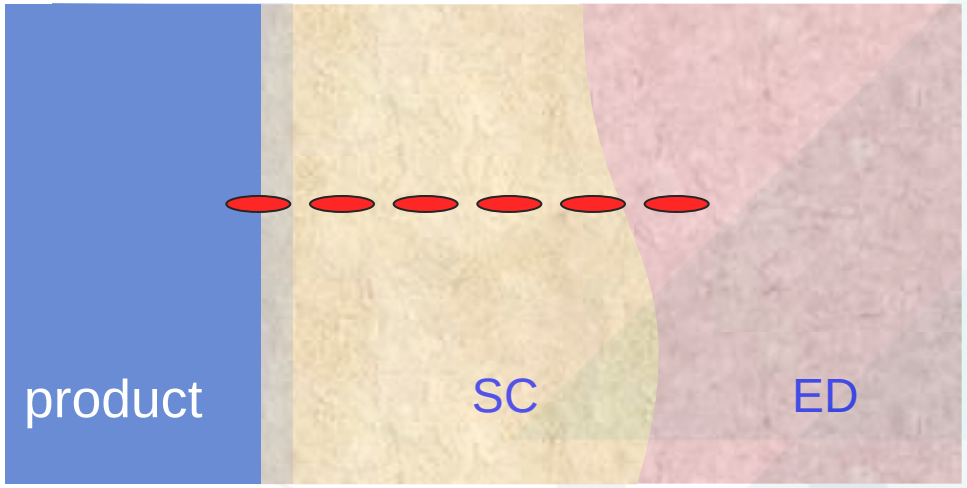
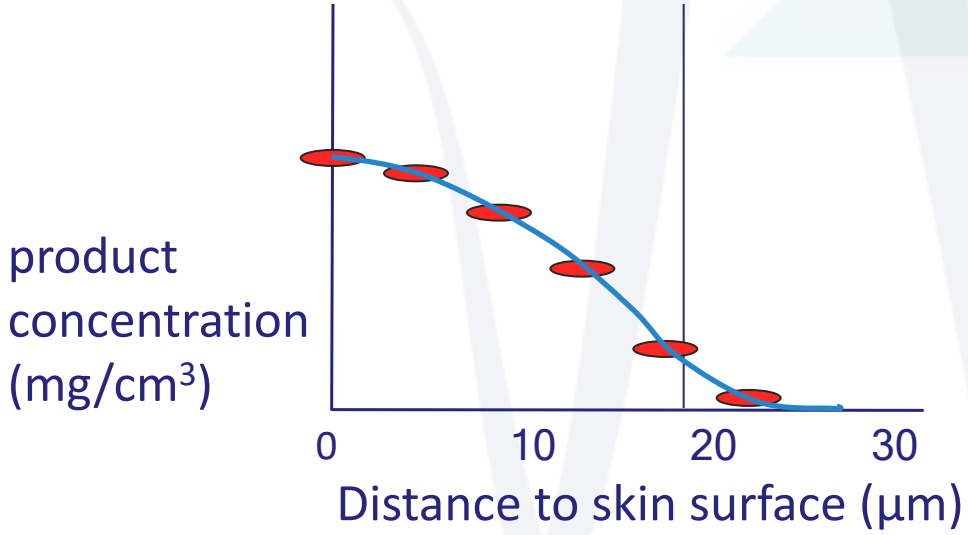
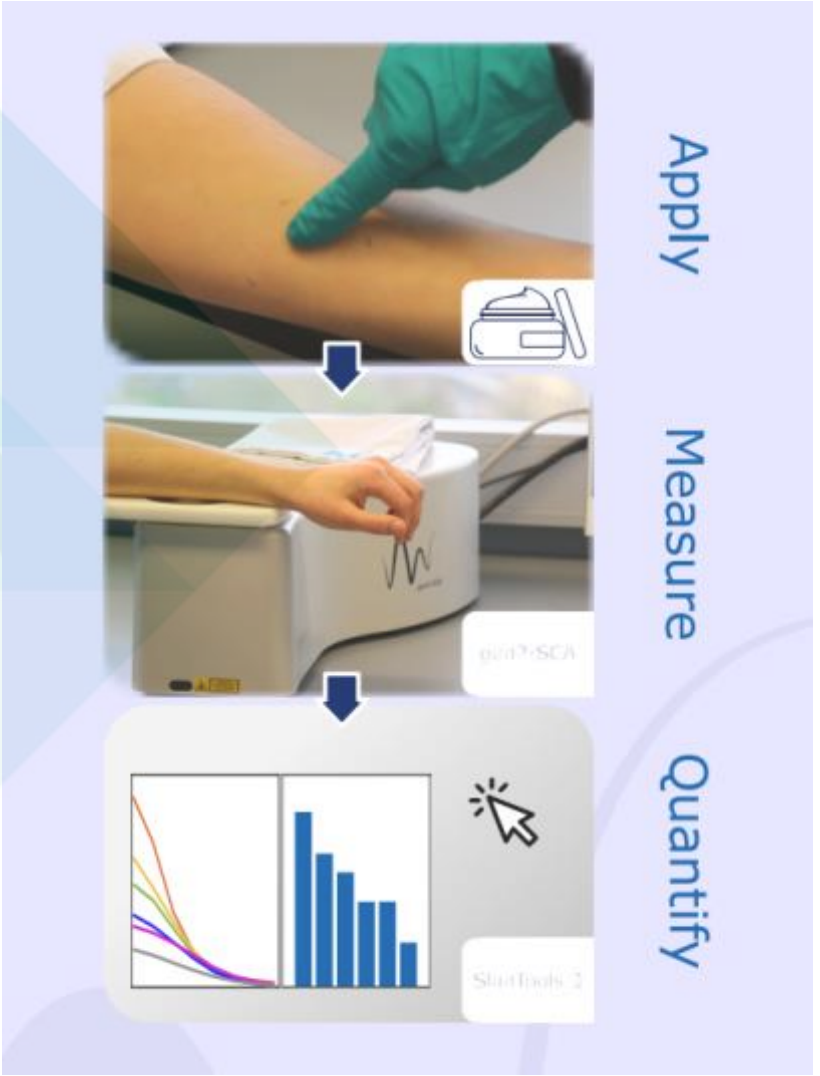
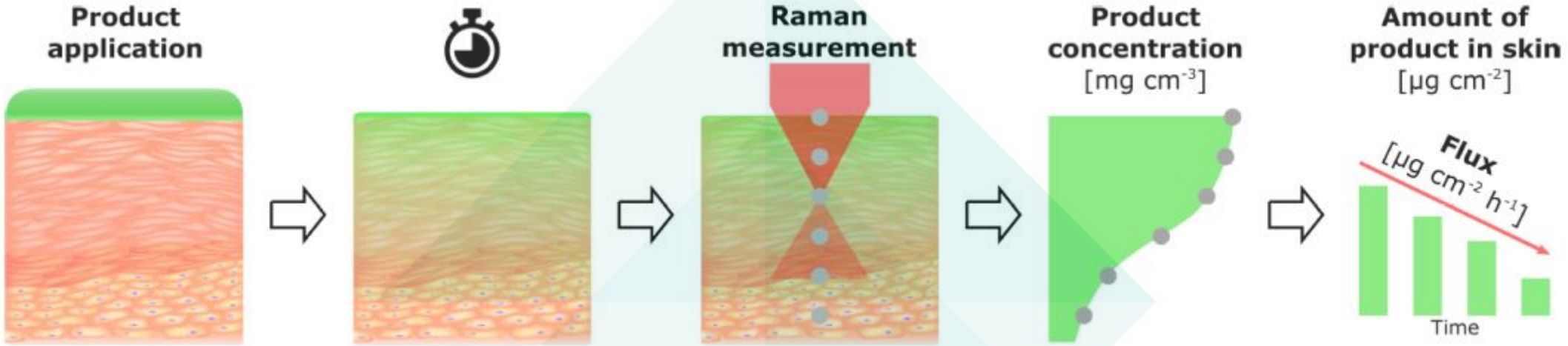
Skin penetration of chemicals: quantitative *in vivo* analysis



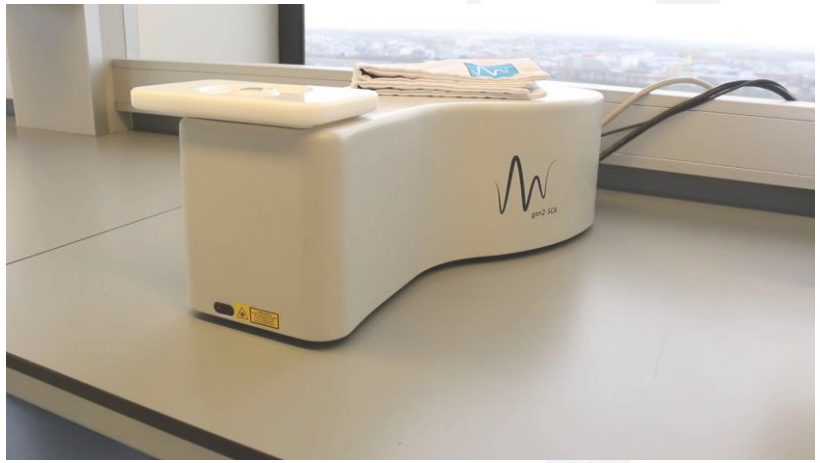




In vivo skin penetration analysis



- 1 minute /profile
- 6-10 profiles per time point

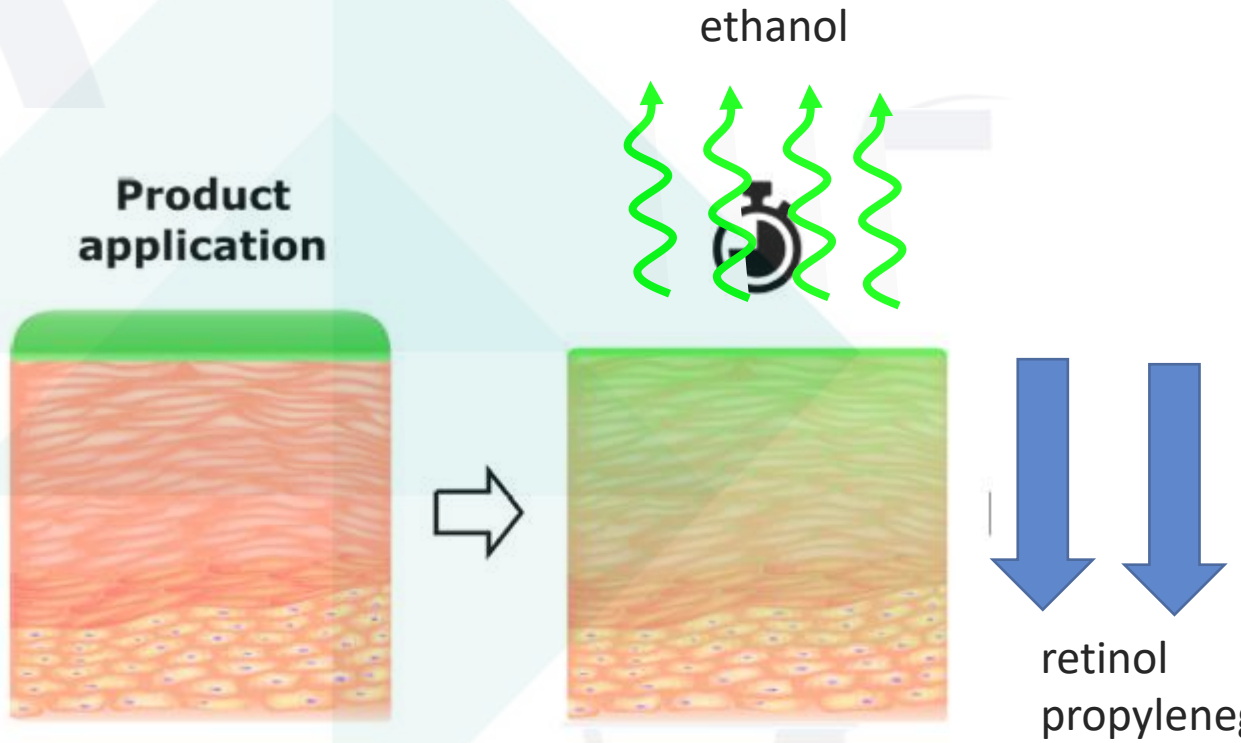


gen2-SCA → **SkinTools 3**

Caspers *et al.* Translational Biophotonics (2019), https://doi.org/10.1002/tbio.201900004

Example: retinol & propylene glycol

- 0.3% retinol in 70% ethanol – 30% PG
- Volar forearm
- 70 µl on skin area of 4 x 4 cm^2
- 10 min



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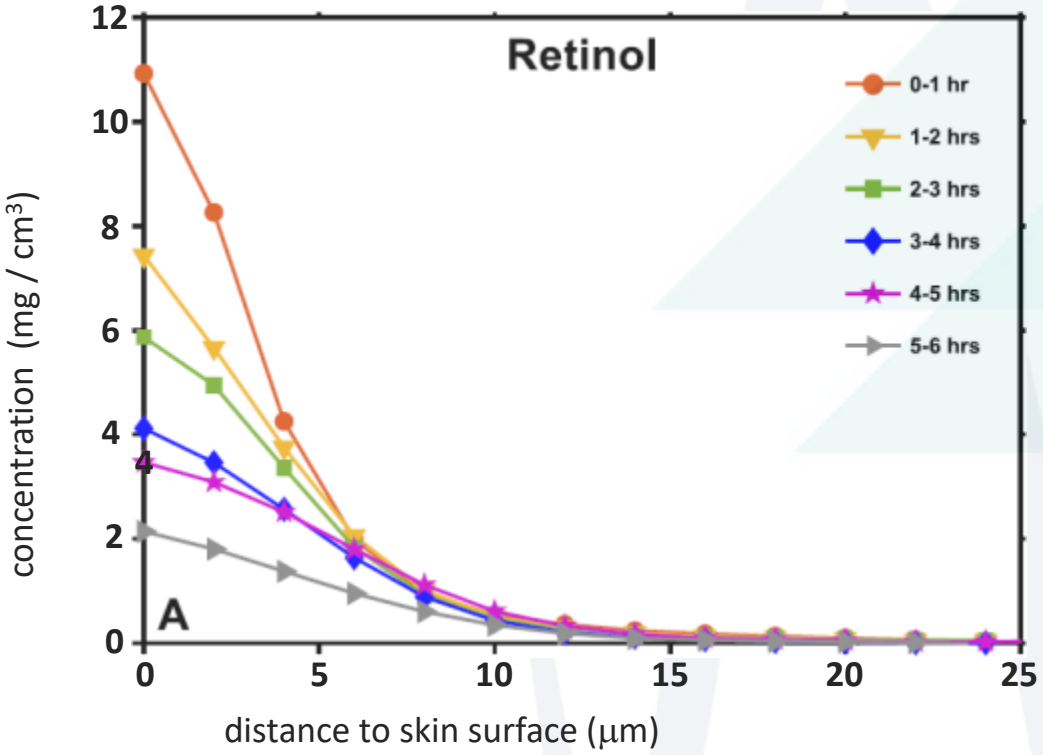
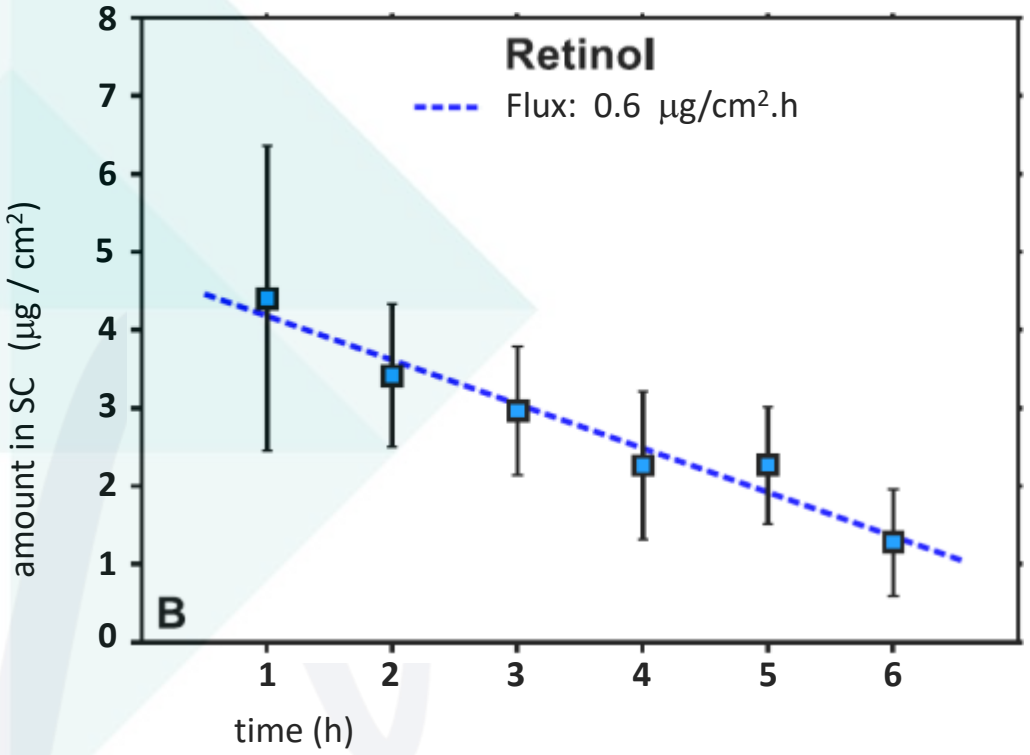
Skin penetration: retinol & propylene glycol





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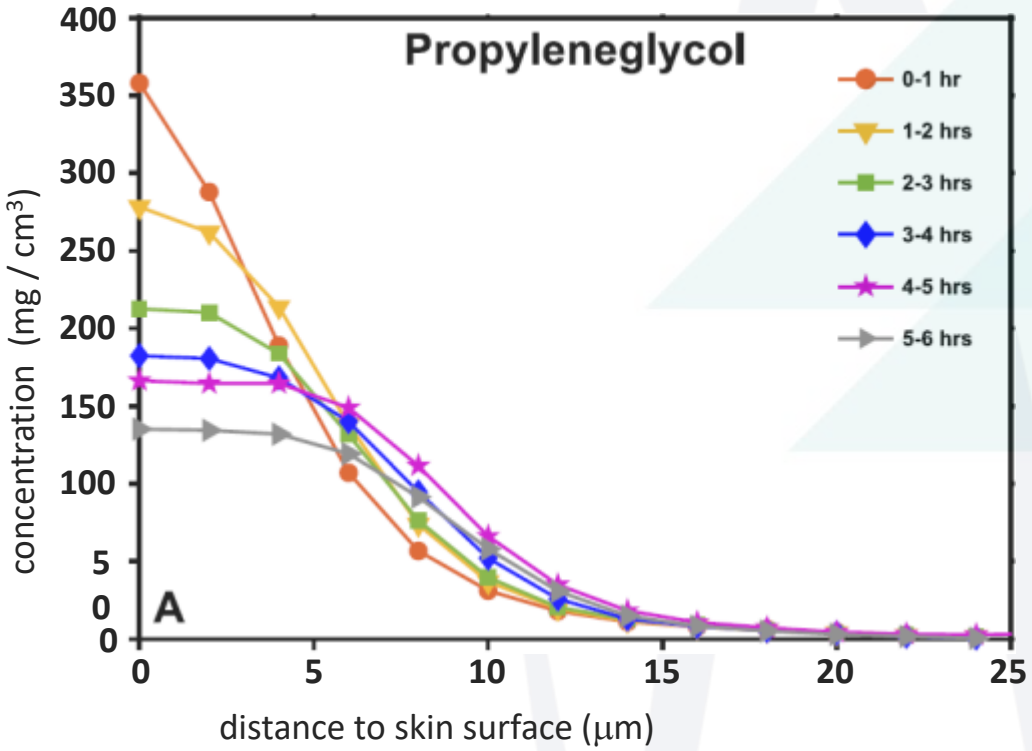
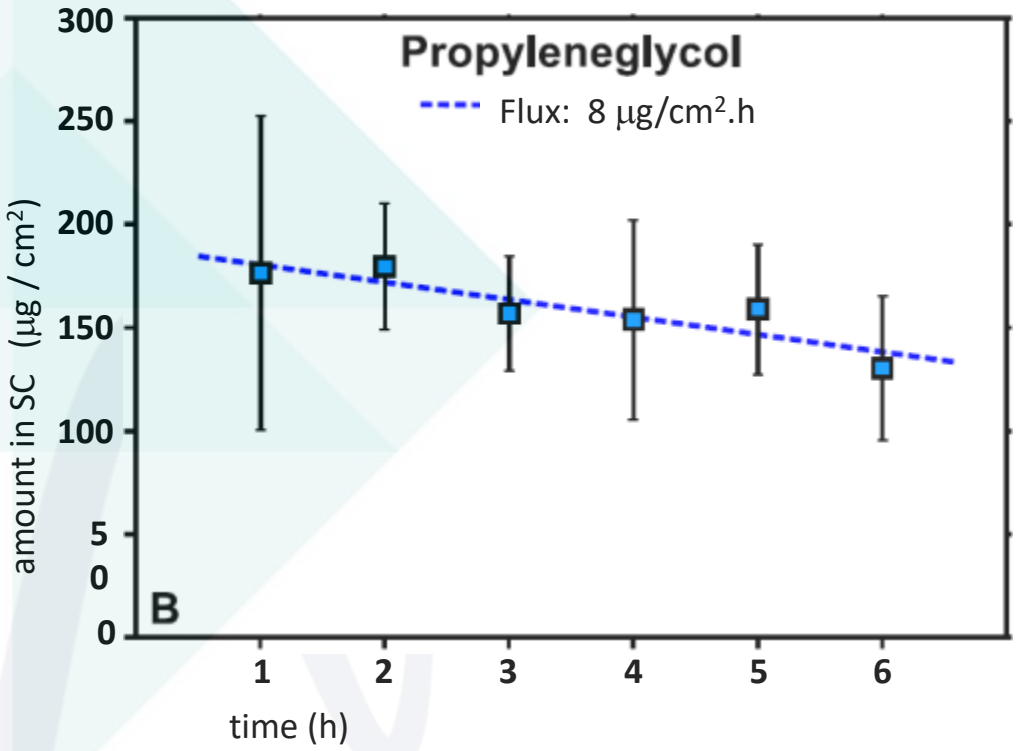
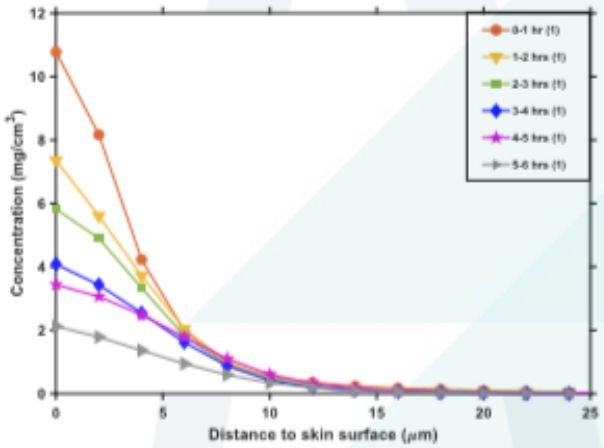


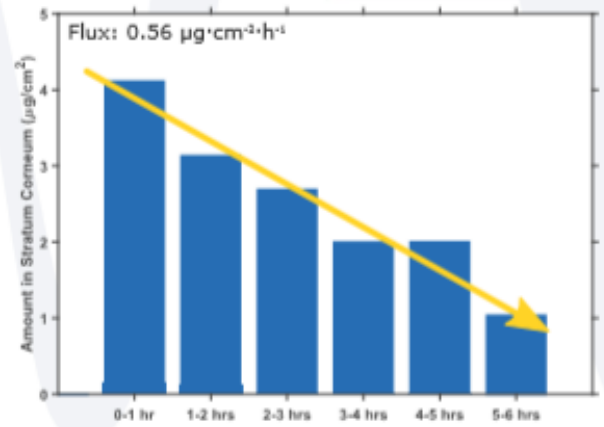


Caspers *et al.* Translational Biophotonics (2019), https://doi.org/10.1002/tbio.201900004

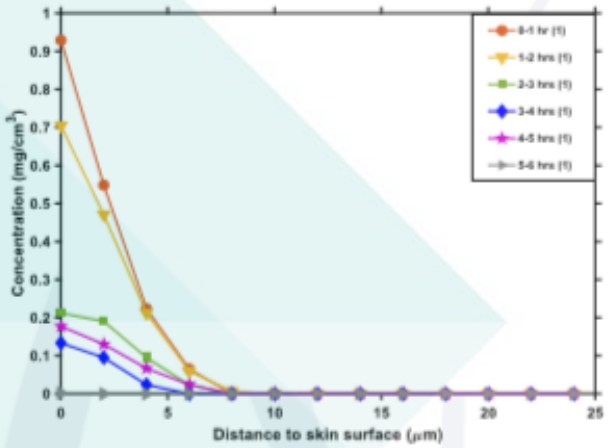
Skin penetration: retinol – effect of formulation

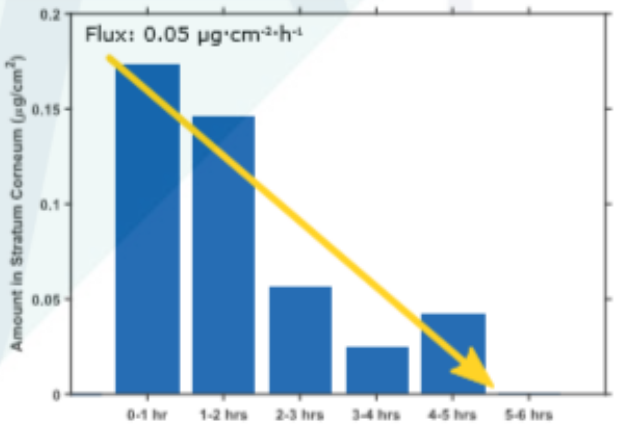
0.3 % Retinol	30 : 70 Propyleneglycol : Ethanol





0.3 % Retinol	Myritol (Caprylic acid)





10 times lower penetration

SkinTools 3 library of calibrated materials

Material	Use
Caffeine	API
Hydrocortisone	API
Ibuprofen	API
Lidocaine	API
Salicylic Acid	API
Testosterone	API
Hydroquinone	API
L-Ascorbic Acid	API
Nicotine	API
Diclofenac	API
Glycolic acid	API

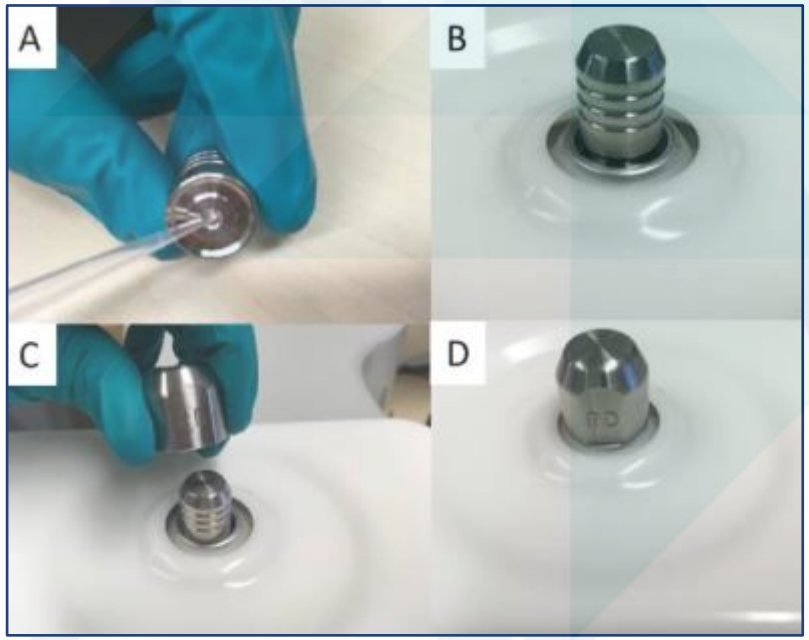
Material	Use
Acetone	Excipient
Glycerol	Excipient
Oleic Acid	Excipient
Propane-Diol	Excipient
Propylene Glycol	Excipient
Ethanol	Excipient
Butylen Glycol Dicaprilate	Excipient
Myritol	Excipient
1-Propanol	Excipient
Benzaldehyde	Excipient
Jojoba Oil	Excipient
2-octyl-1-dodecanol	Excipient
1-Tetradecanol	Excipient
Sodium Dodecyl Sulfate	Excipient
Butylene glycol	Excipient
2-Butanone	Excipient

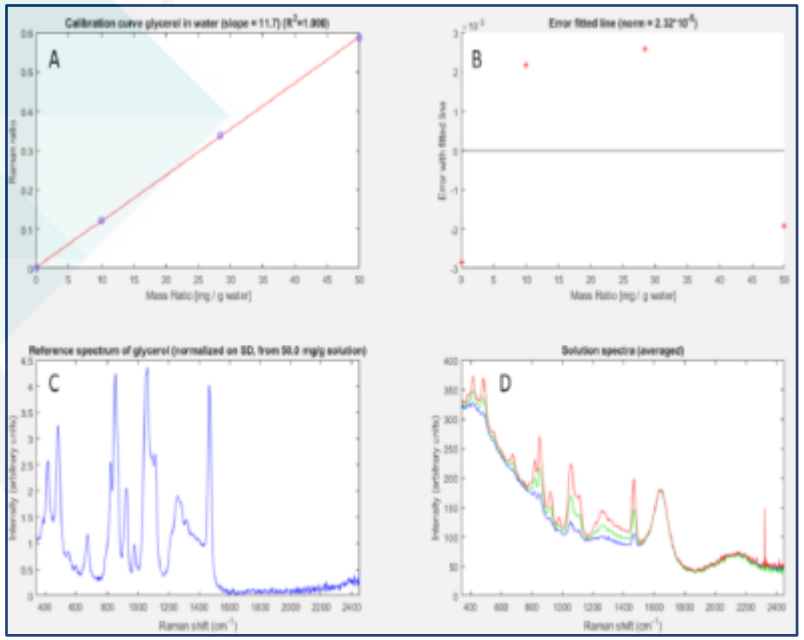
Material	Use
3-Methylsalicylic Acid	Fragrance
Methyl Salicylate	Fragrance
Benzyl Alcohol	Fragrance
Coumarin	Fragrance
4-Methylsalicylic Acid	Fragrance
Benzoin	Fragrance
Vanillin	Fragrance
Geraniol	Fragrance
Menthol	Fragrance
Phenethyl Alcohol	Fragrance
Laurocapram	Penetration enhancer
DMSO	Penetration enhancer
Citric Acid	Preservative
Methylparaben	Preservative
Propylparaben	Preservative
Isopropanol	Preservative
Sodium Benzoate	Preservative
Bisfenol A	Preservative
Levulinic Acid	Preservative
2-phenoxy ethanol	Preservative
Benzoic Acid	Preservative
Sorbic Acid	Preservative
Ethylparaben	Preservative
Isobutylparaben	Preservative

Material	Use
α-Tocopherol	Skin Conditioning Agent
Adenosine	Skin Conditioning Agent
Glycine	Skin Conditioning Agent
L-Alanine	Skin Conditioning Agent
Nicotinamide	Skin Conditioning Agent
PCA	Skin Conditioning Agent
Retinol	Skin Conditioning Agent
Serine	Skin Conditioning Agent
Squalane	Skin Conditioning Agent
Oxybenzone	UV Filter
Uvinul A Plus	UV filter
Avobenzone	UV filter
Ethylhexyl triazone	UV filter
Tinsorb S	UV filter

Typical limits of detection:
~ 0.1 – 1 mass%

SkinTools 3 library … add your own materials





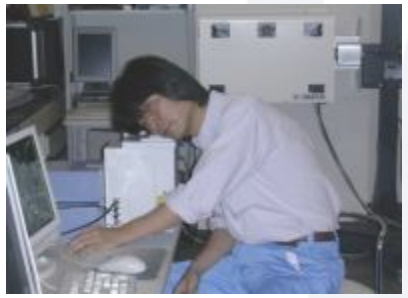
Accessible body areas - examples

leg (calf)

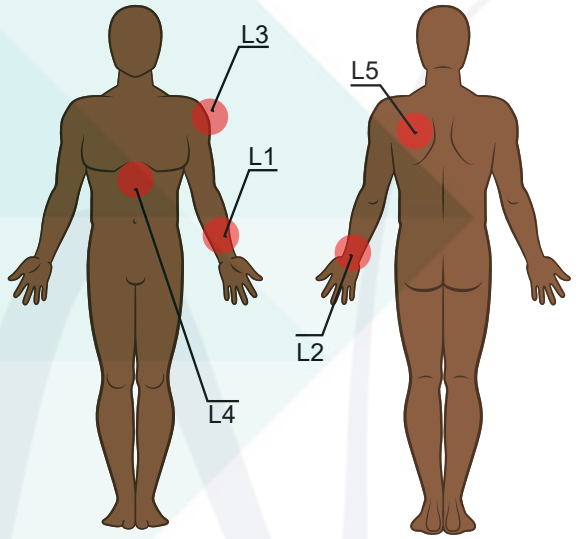
axilla

forehead

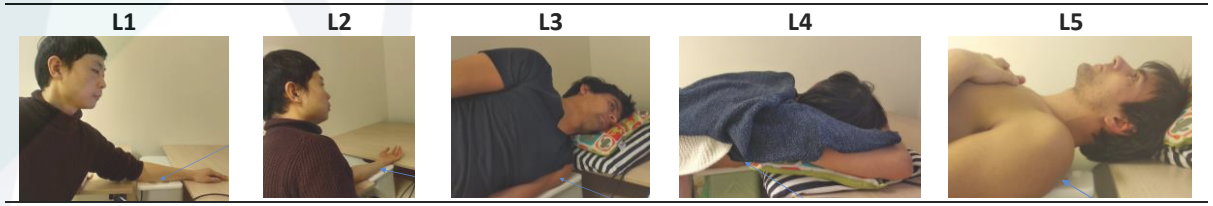
lip



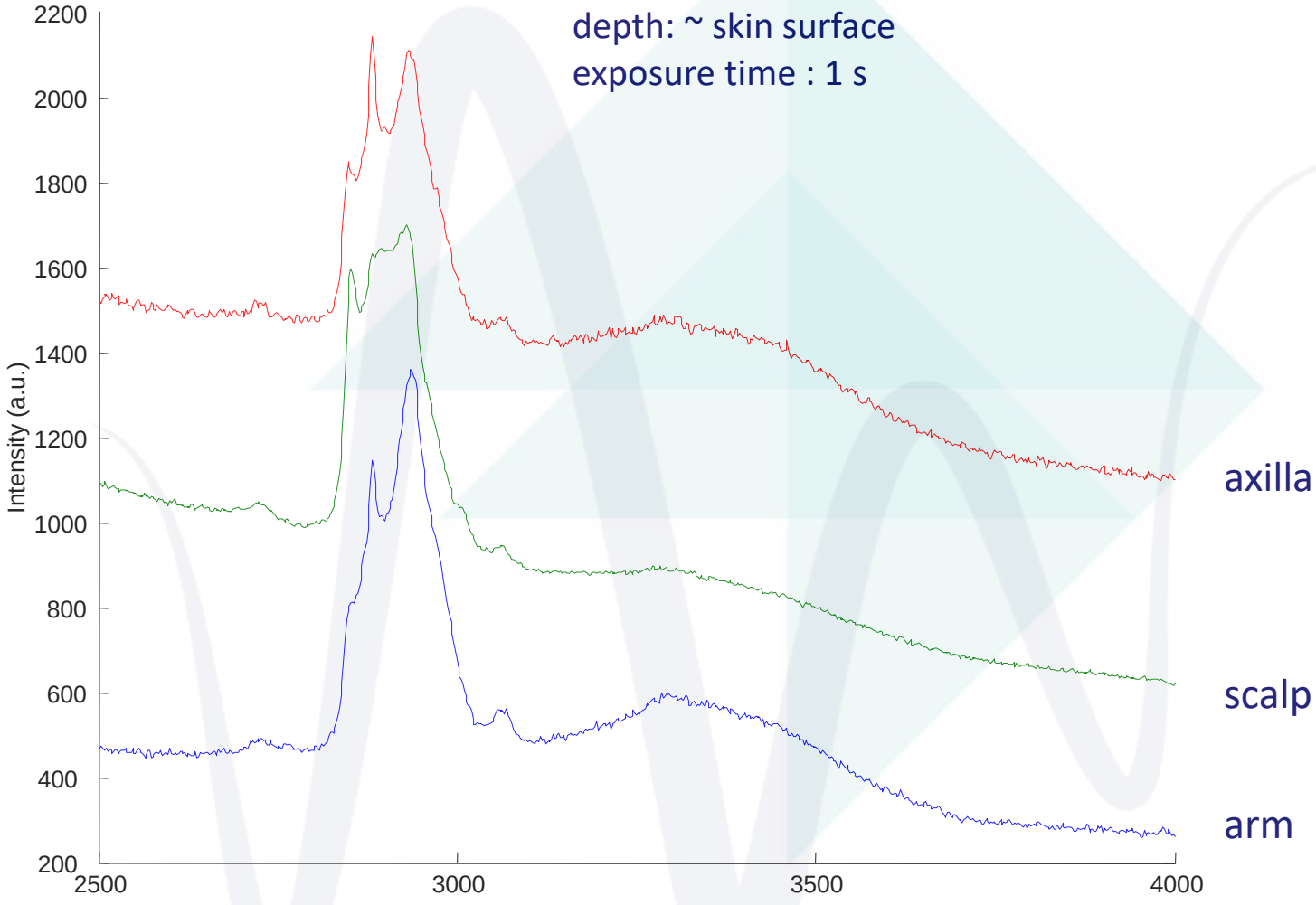
cheek



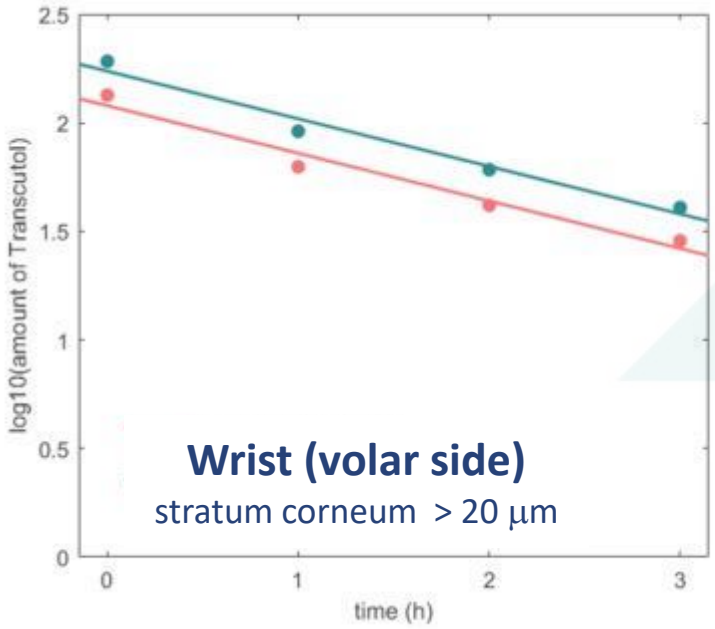
L1	Anterior forearm
L2	Posterior wrist
L3	Posterior arm
L4	Thorax (rib cage)
L5	Back dorsal

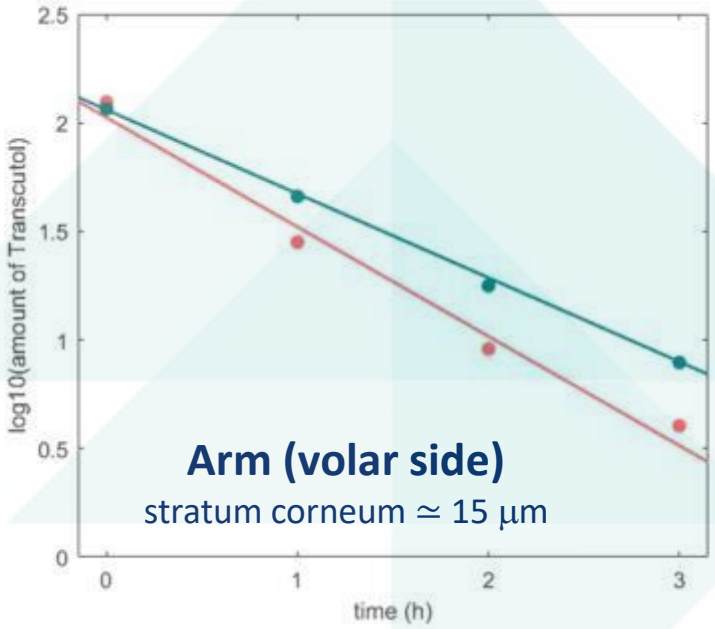


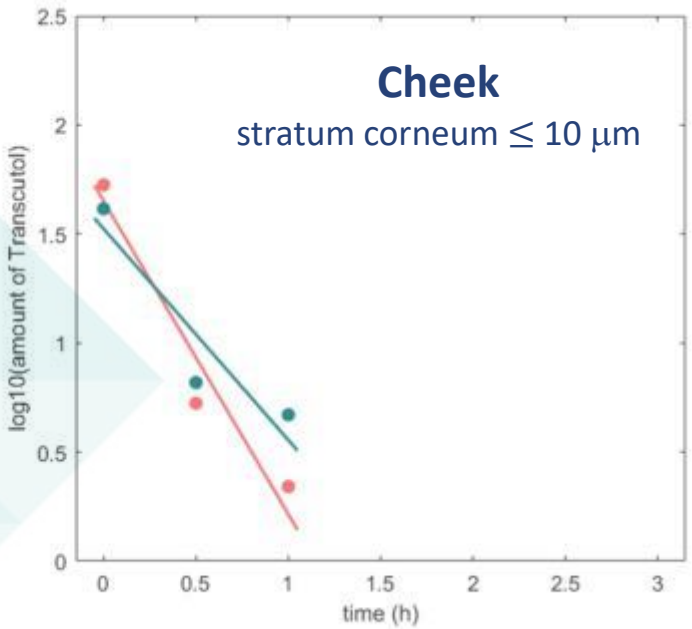
Arm, scalp and axilla compared



Human skin: Transcutol experiments







- Van der Bend – patch with 15 µl Transcutol
- 30 minutes application
- Skin surface wiped clean before Raman measurements
- 2 subjects (--- ---)

unpublished

Wrap-up

- **gen2-SCA + SkinTools 3 for**
  - non-invasive analysis of molecular composition of the skin
  - quantitative analysis of the penetration of topical products
  - simultaneous analysis of actives and vehicles/excipients
    - optimisation of formulations
    - insight in mode of penetration
- **Limit of detection: ~ 0.1-1 mass%**
  - molecule dependent: e.g. some UV-filters LoD ~ 0.01 mass%
- **skin ≠ skin:**
  - differences in molecular composition of the skin
  - *in vivo* skin penetration/permeation dependent on anatomical location.

proderm

proderm Webinar: Raman Spectroscopy in dermatology trials

26. April 2022

Stephan Bielfeldt

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www.proderm.de

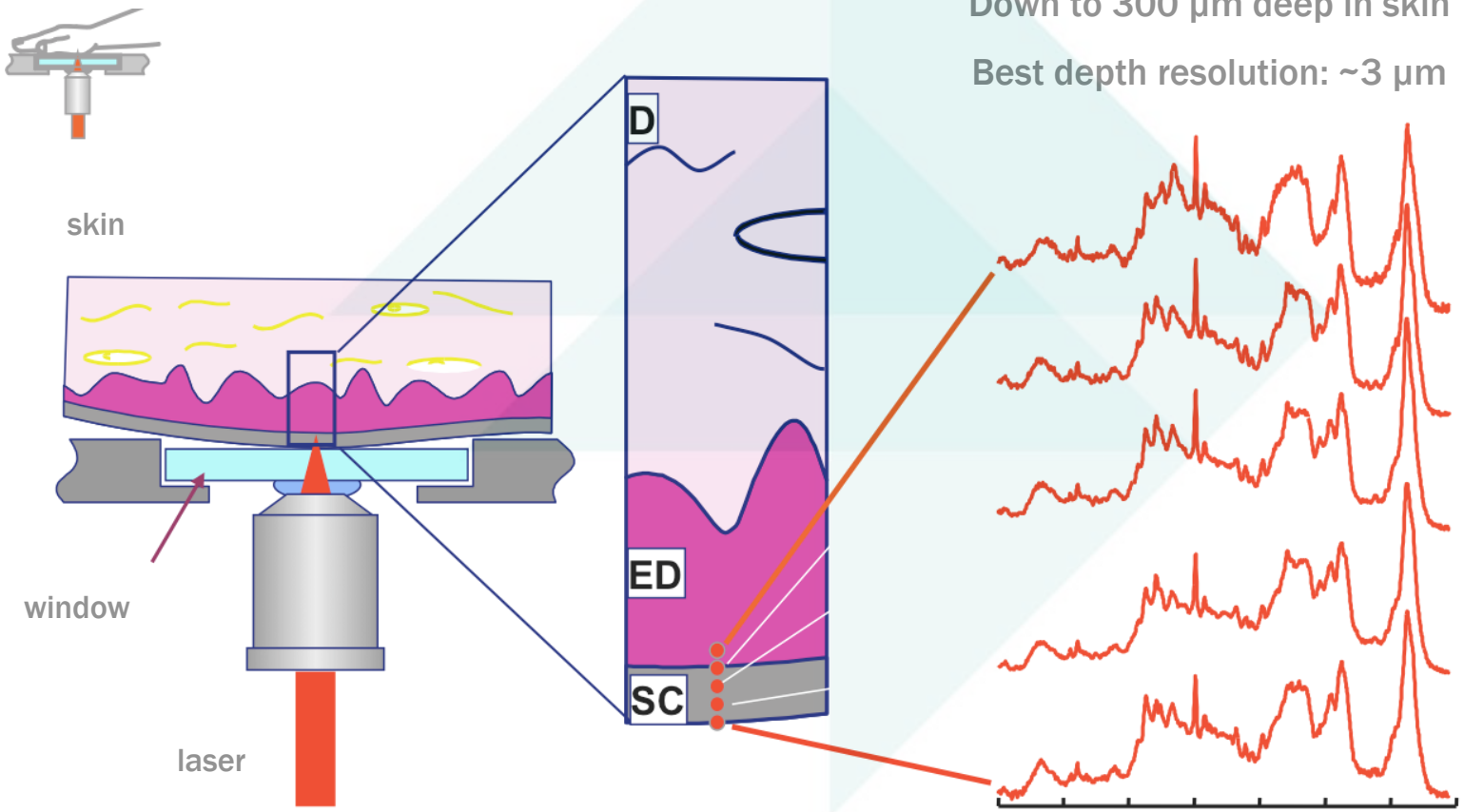
Our ambition? **Excellence.**

Agenda

- Confocal Raman Spectroscopy on skin, how does it work?
- Measurement of water in the skin
- NMF and skin lipids: Molecules crucial for an intact barrier
- Measurement of skin penetration and permeation
- Summary and conclusions

In Vivo Measurement of Raman Spectra at Different Skin Depths

Inverse Confocal Microscopy



Characteristics of the Raman device (2nd Generation)

- "gene2-SCA Ultimate" manufactured by RiverD International B. V., Rotterdam, Netherlands

- built-in two lasers (671 nm and 785 nm)
- Fingerprint (FP): 400 – 1800 cm^{-1}
- High Wave Number (HWN): 2500 – 3800 cm^{-1}

- Movable table in two dimensions
- Adjustable pinholes: 25, 50 and 100 µm
- Resolution: 3, 5 and 10 µm

- Typical measurement times:
- FP spectrum: 5 sec.
- HWN spectrum: 1 sec.

Agenda

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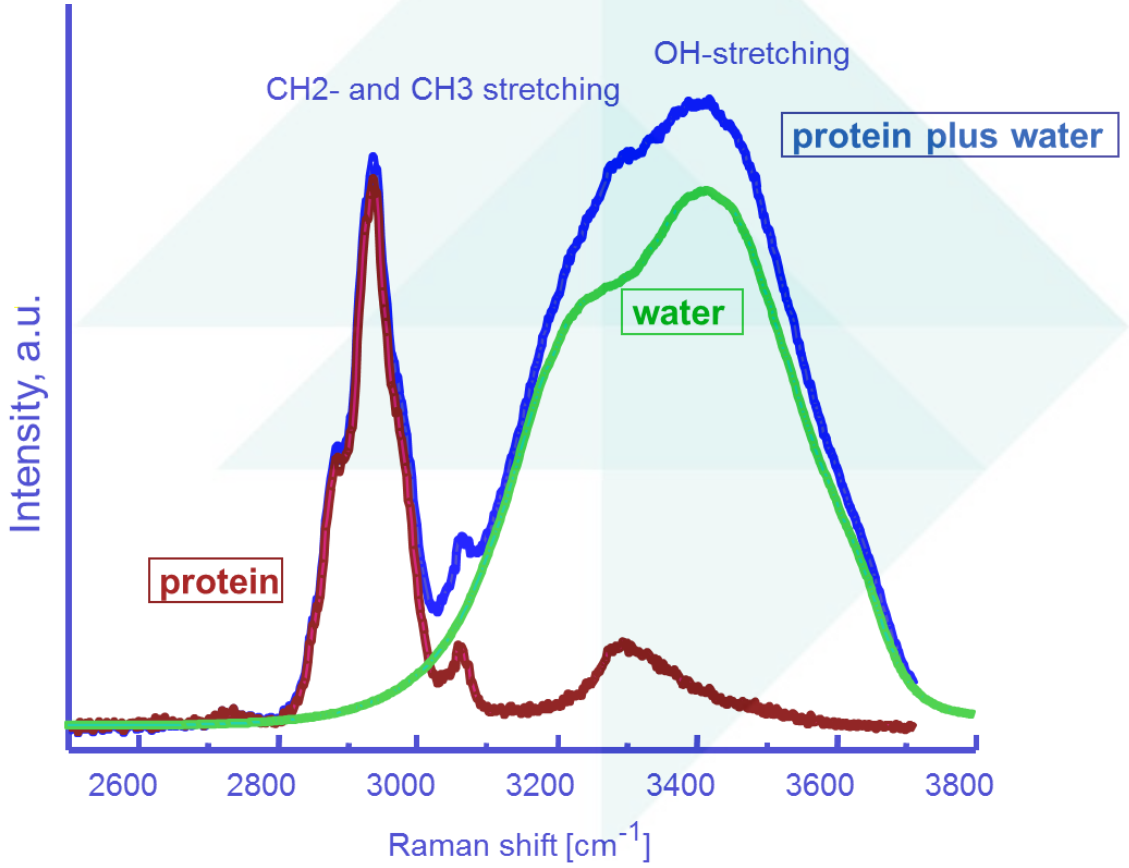
Water Measurement with Confocal Raman Spectroscopy

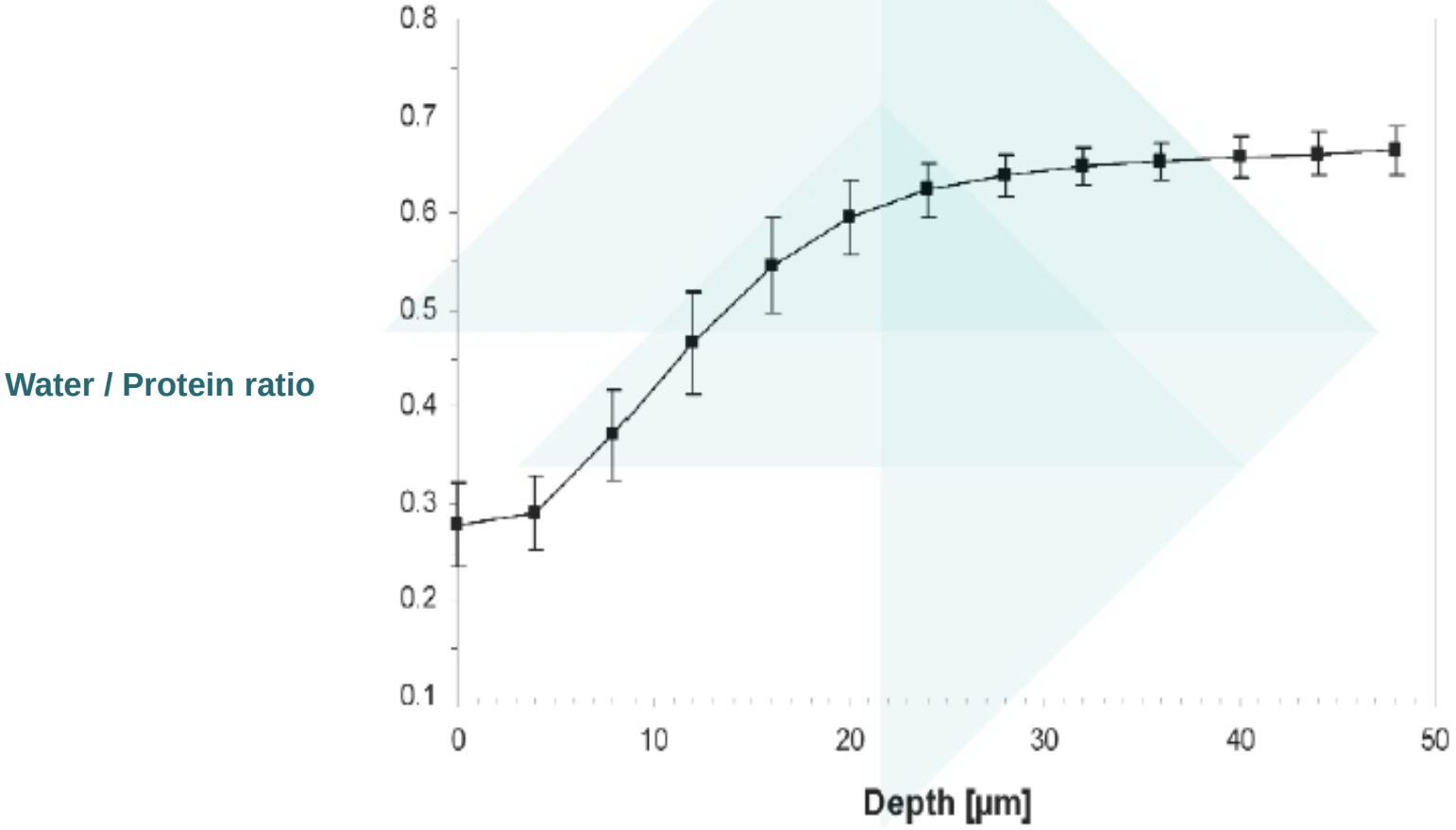
High Wave Number Spectra of the Skin



Incident Laser Light of 671 nm

Water Measurement with Confocal Raman Spectroscopy

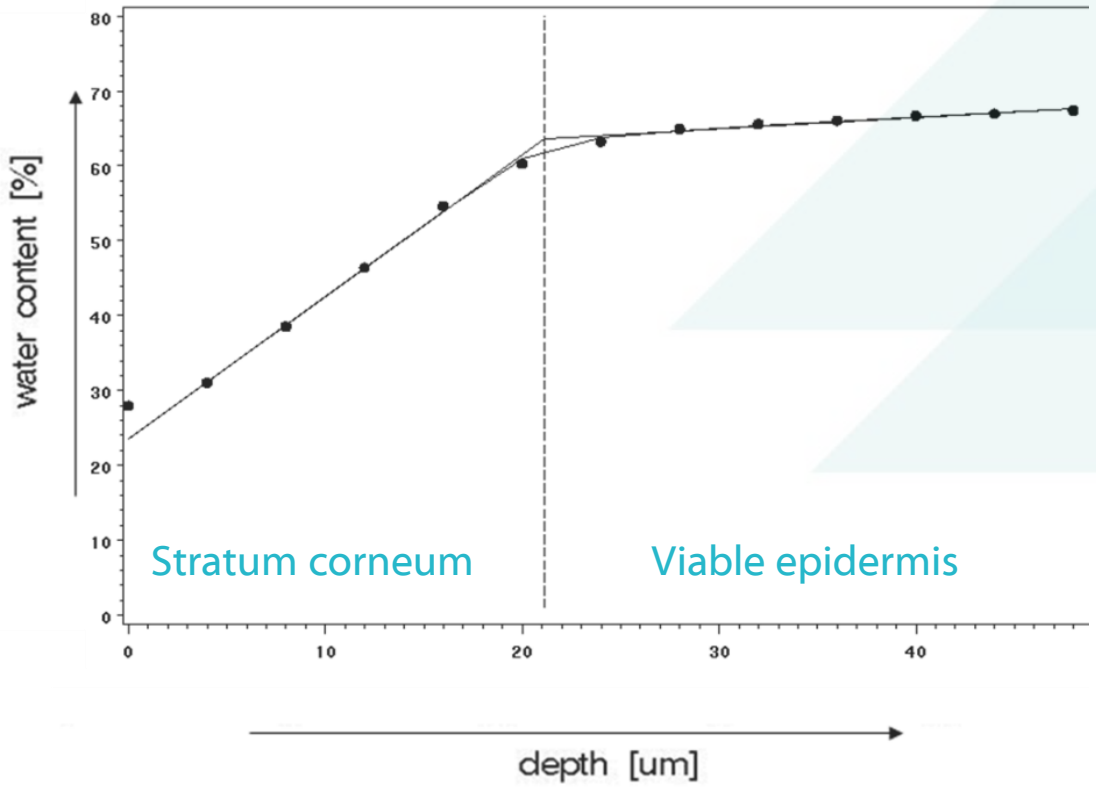
Assessment of Profiles Across SC and Viable Epidermis

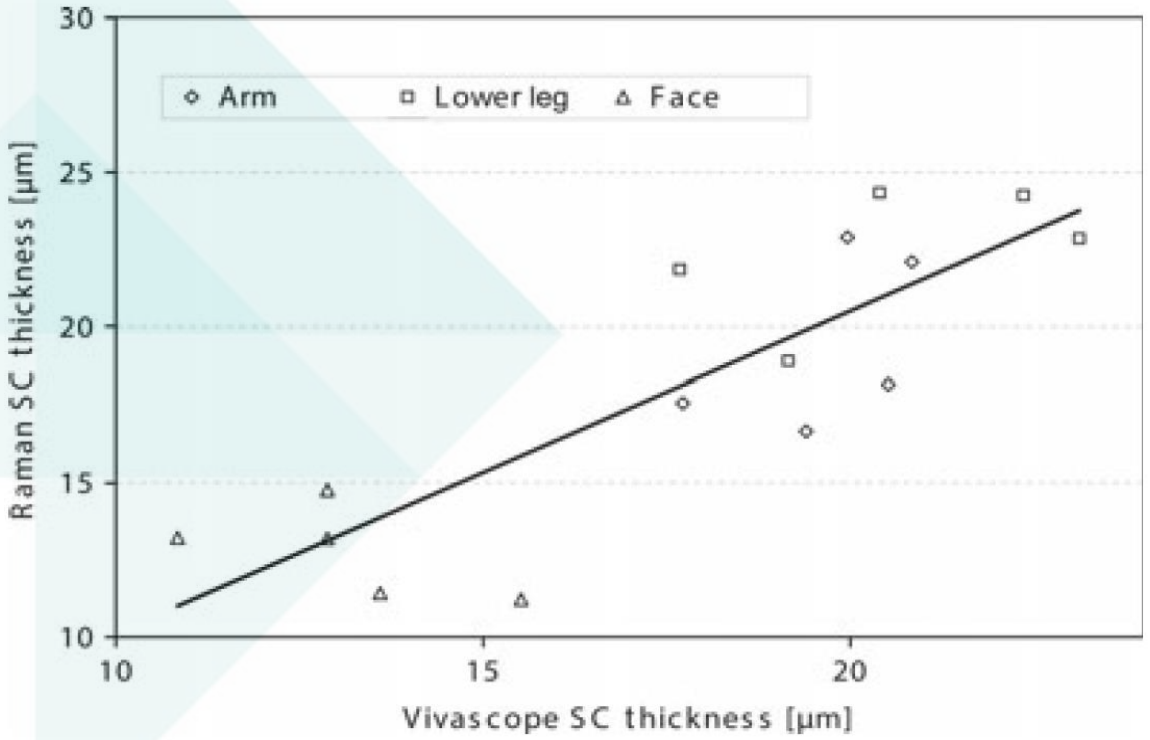


Water profile of 1 subject on volar forearm (10 repetitions)

Validation of Confocal Raman Spectroscopy

Depth Detection: Fit Model to Detect the SC Border with CRM is Confirmed with Confocal Reflectance Microscopy



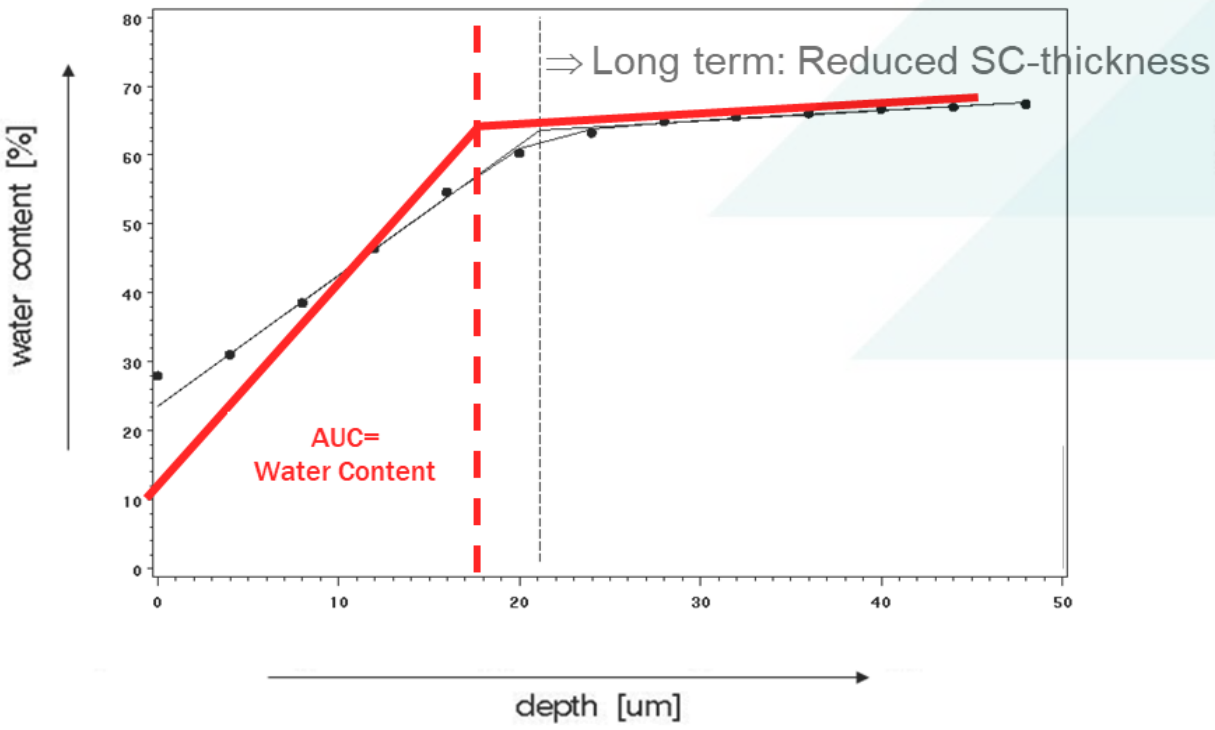


Böhling, A., Bielfeldt, S., Himmelmann, A., Keskin, M., & Wilhelm, K. P. (2014). Comparison of the stratum corneum thickness measured in vivo with confocal Raman spectroscopy and confocal reflectance microscopy. Skin Research and Technology, 20(1), 50-57.

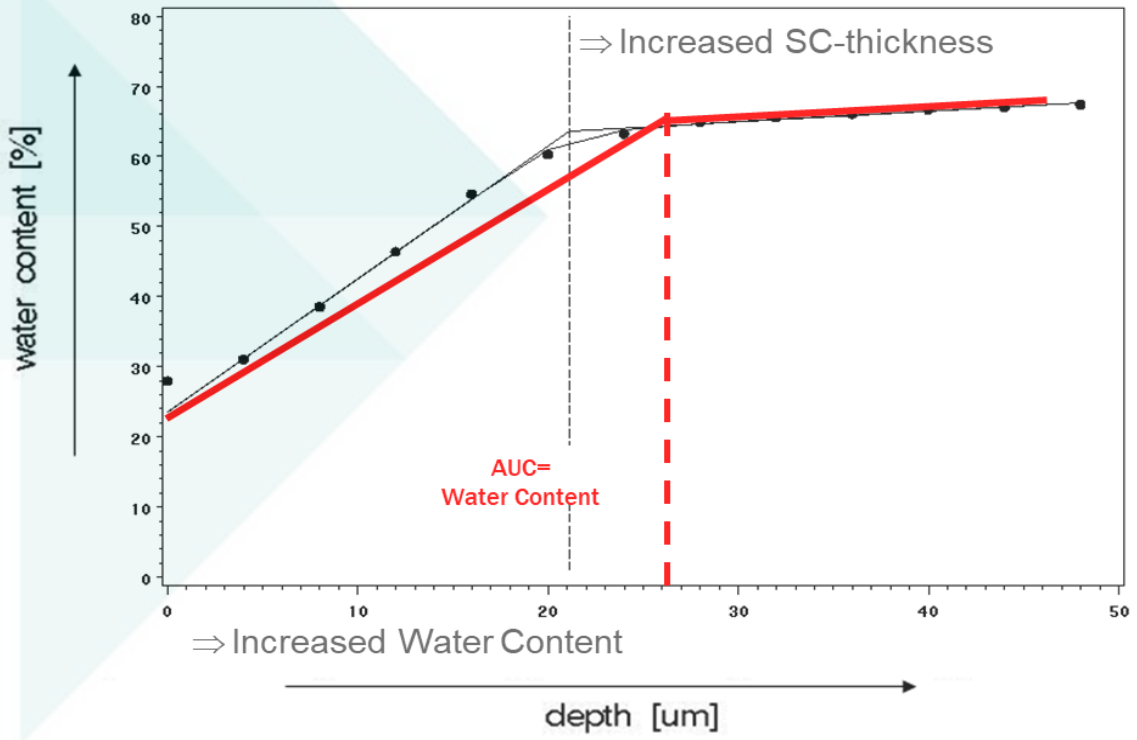
Water Profiles: Example of Moisturizer and Emollient Effects

Two Observations: Increase of Water Gradient and SC Swelling

Long term moisturizer effect (Glycerol 10%)



Short term emollient effect



Agenda

- Confocal Raman Spectroscopy on skin, how does it work?
- Measurement of water in the skin
- NMF and skin lipids: Molecules crucial for an intact barrier
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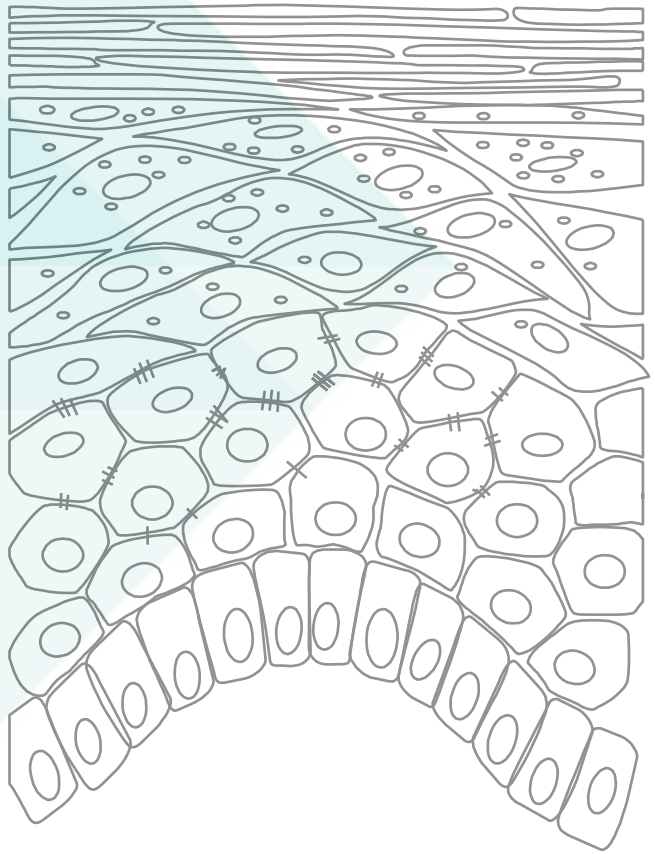
- Summary and conclusions

Which Skin Components Contribute to the Raman Spectrum in the Epidermis?

- Keratin (ca. 70% of dry wt.)
- Water (20-70% of total wt.)
- **NMF (20-30% of dry wt.)**
  - Free Amino Acids 40%
  - PCA 12%
  - Lactate 12%
  - Urea 7%
- **Lipids**
  - Cholesterol
  - Ceramides / Free Fatty Acids

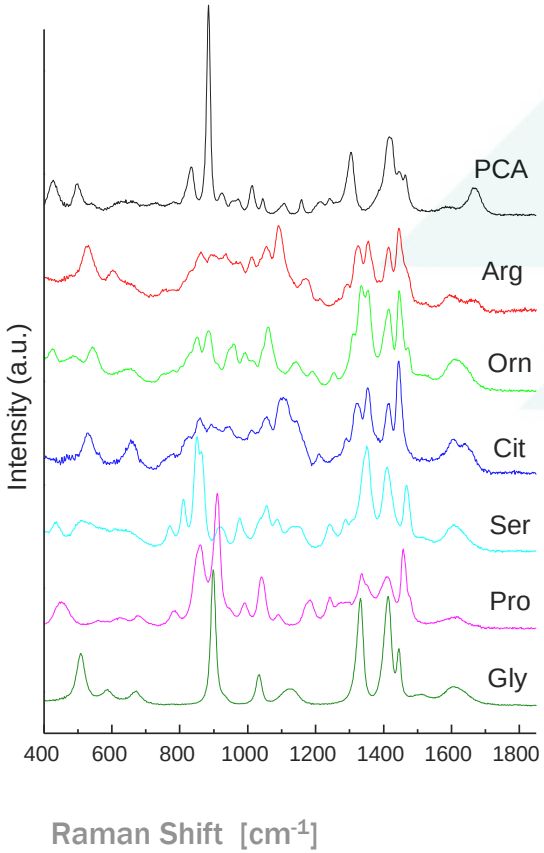


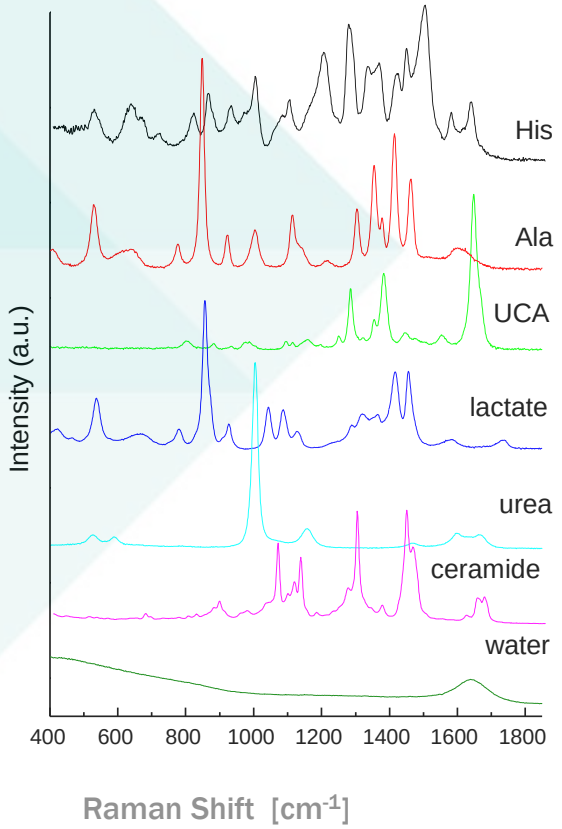
Caspers, P.J. In vivo skin characterization by confocal Raman microspectroscopy. (2003)

Raman Spectrum of Skin: A Complex overlap of Spectra

Multiple Components Analysis and Advanced Fit Model applied

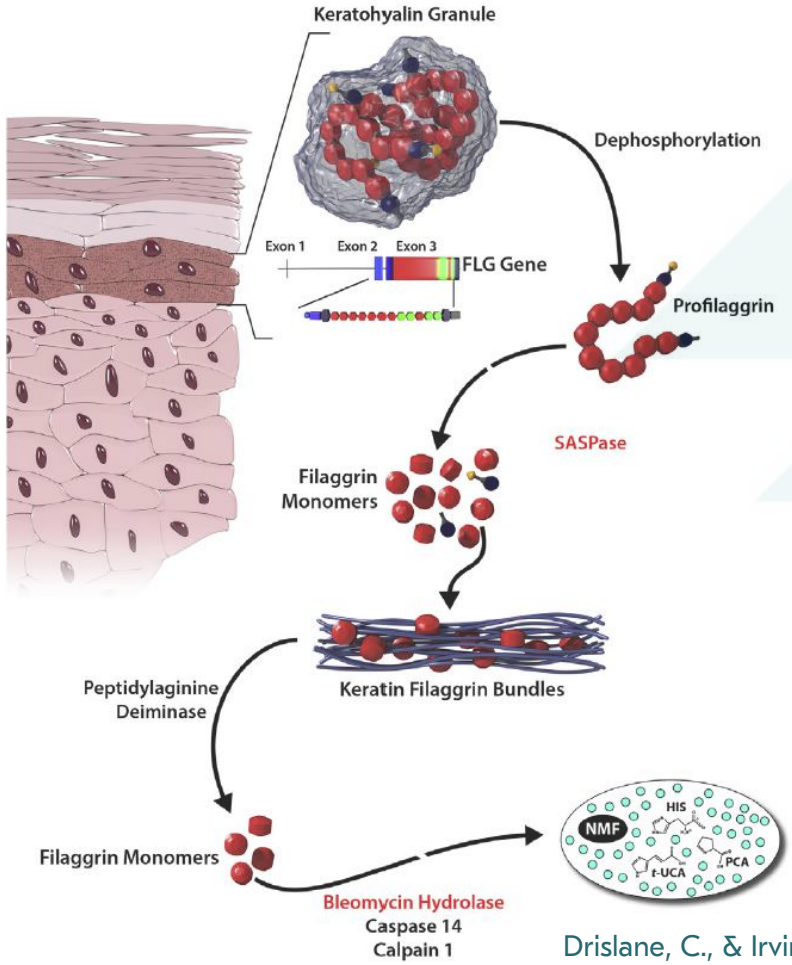
Single Fingerprint spectra of main skin components

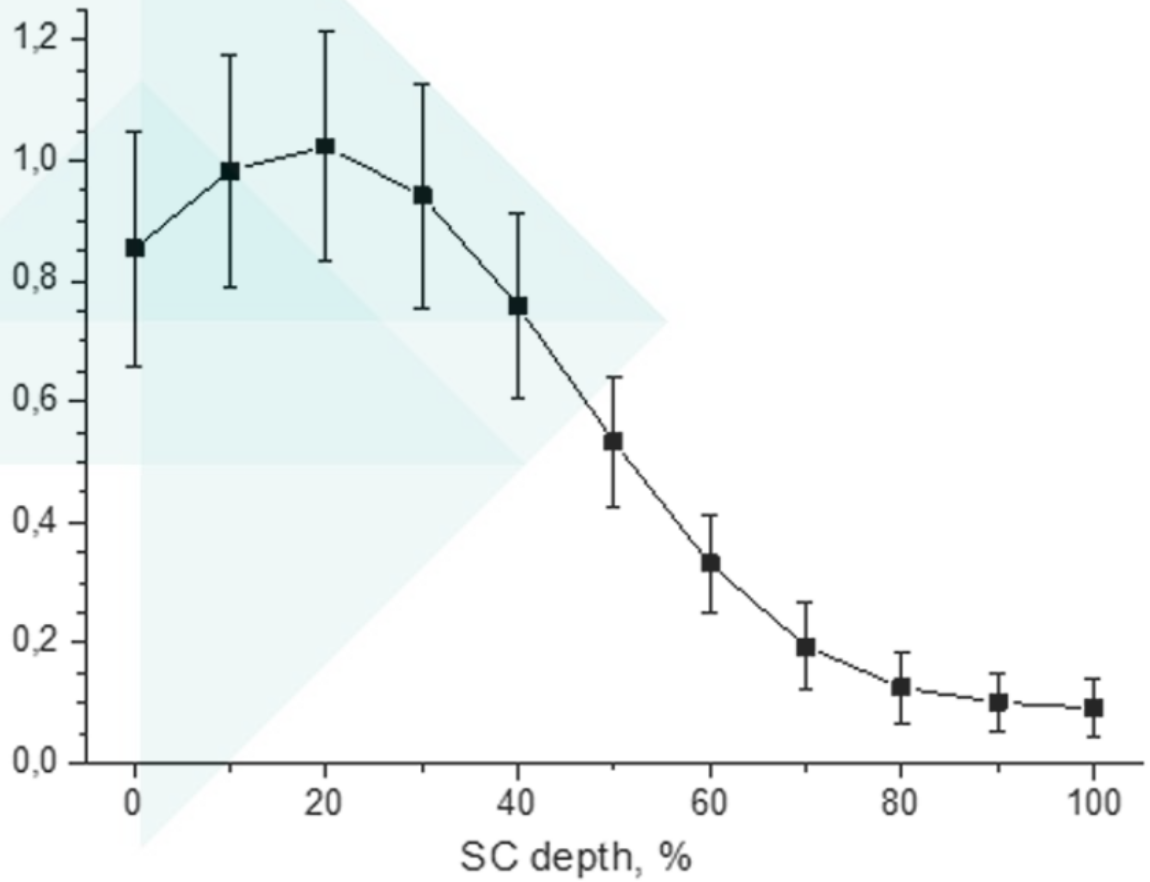




From Filaggrin to Natural Moisturization Factor (NMF)

Right side: The typical Distribution of Total NMF in SC as measured with CRS

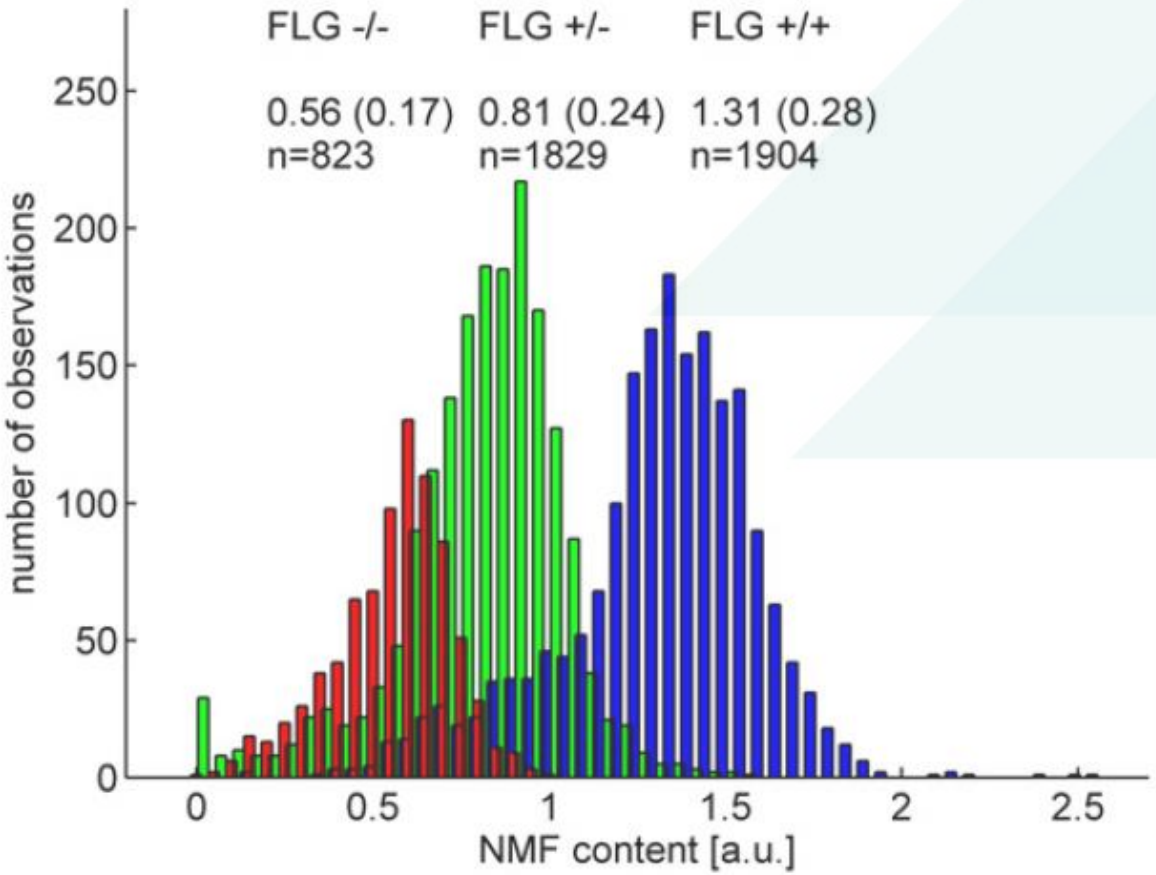




Drislane, C., & Irvine, A. D. (2020). The role of filaggrin in atopic dermatitis and allergic disease. Annals of Allergy, Asthma & Immunology, 124(1), 36-43.

NMF Content as a Marker for Filaggrin Mutations in Atopic Dermatitis

Comparison of two Allel, one Allel, and no Filaggrin Mutations



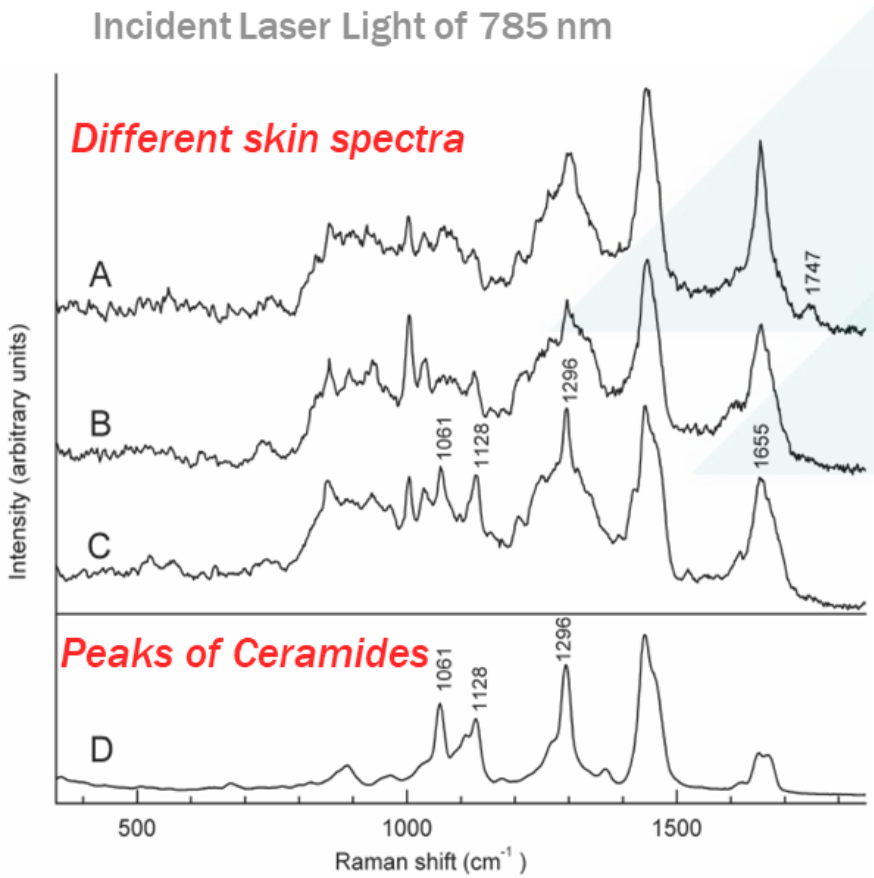
Low NMF content cannot be completely explained by mutations of the Filaggrin gene!

Subject with dry skin but no acute eczema can show a very low NMF-content!

O'Regan, G. M. et al (2010). Journal of allergy and clinical immunology, 126(3), 574-580.

Skin Lipids can be assessed in the Finger Print Spectrum

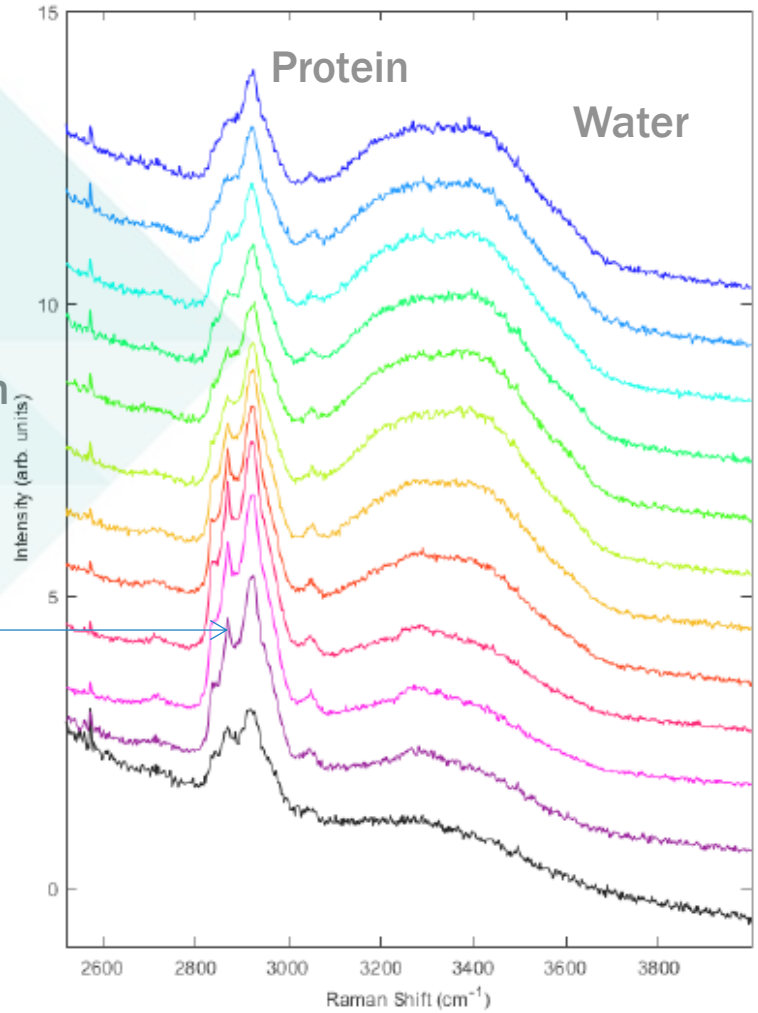
and at High Wave Number (HWN): Lipid to protein ratio



HWN

Incident Laser Light of 671 nm Profiles at different depths in the horny layer

Lipid peak



Caspers, P. J., Lucassen, G. W., Bruining, H. A., & Puppels, G. J. (2000). Automated depth-scanning confocal Raman microspectrometer for rapid in vivo determination of water concentration profiles in human skin. Journal of Raman spectroscopy, 31(8-9), 813-818.

HWN-Measurement of Total Lipids in the Epidermis

Discrimination between Atopic Eczema and healthy skin

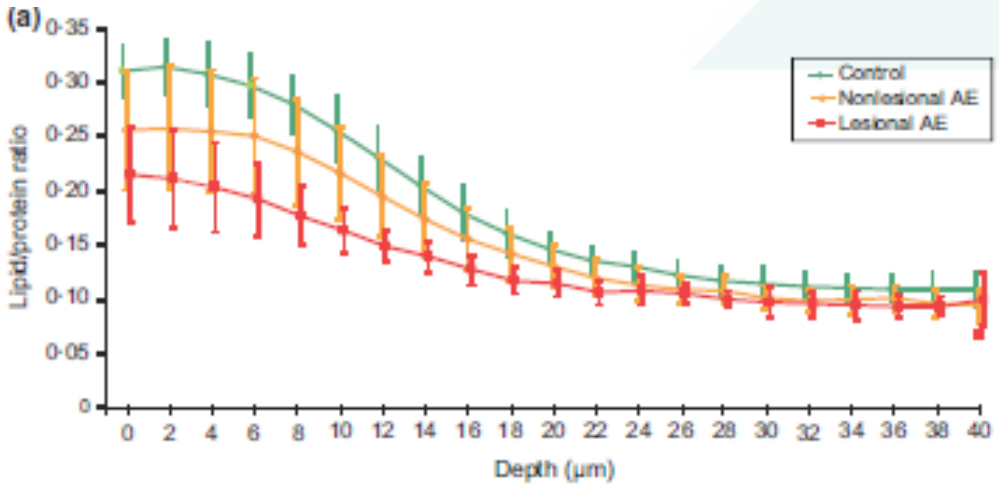
CUTANEOUS BIOLOGY

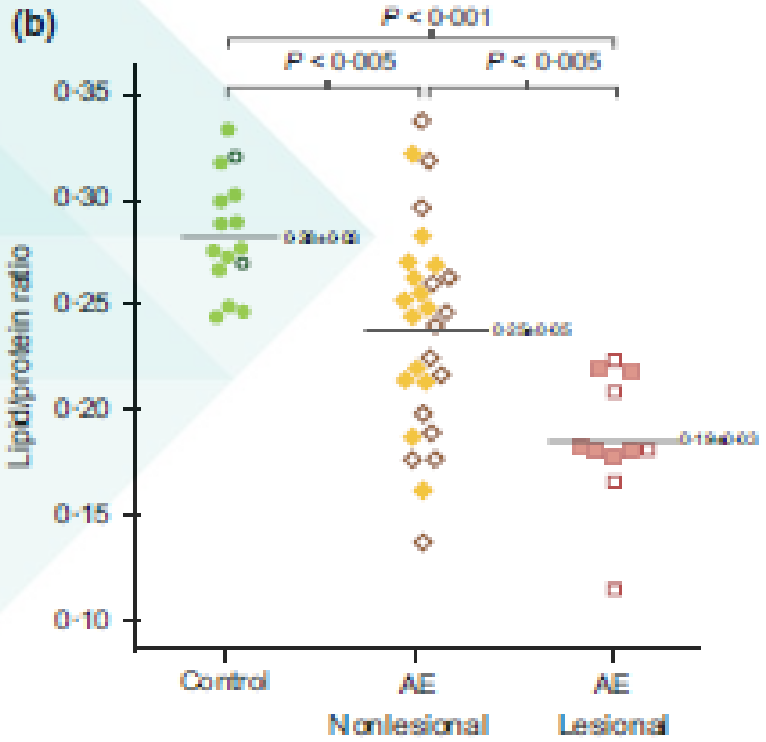
BJD
British Journal of Dermatology

Lipid to protein ratio plays an important role in the skin barrier function in patients with atopic eczema*

M. Janssens,[1] J. van Smeden,[1] G.J. Puppels,[2,3] A.P.M. Lavrijsen,[4] P.J. Caspers[2,3] and J.A. Bouwstra[1]

[1]Department of Drug Delivery Technology, Leiden Academic Centre for Drug Research, Leiden University, Leiden, the Netherlands
[2]Department of Dermatology, Center for Optical Diagnostics and Therapy, Erasmus MC, Rotterdam, the Netherlands
[3]RiverD International B.V., Rotterdam, the Netherlands
[4]Department of Dermatology, Leiden University Medical Center, Leiden, the Netherlands





British Journal of Dermatology (2014) 170, pp1248–1255

Washout of NMF and Lipids from Scalp by Shampooing

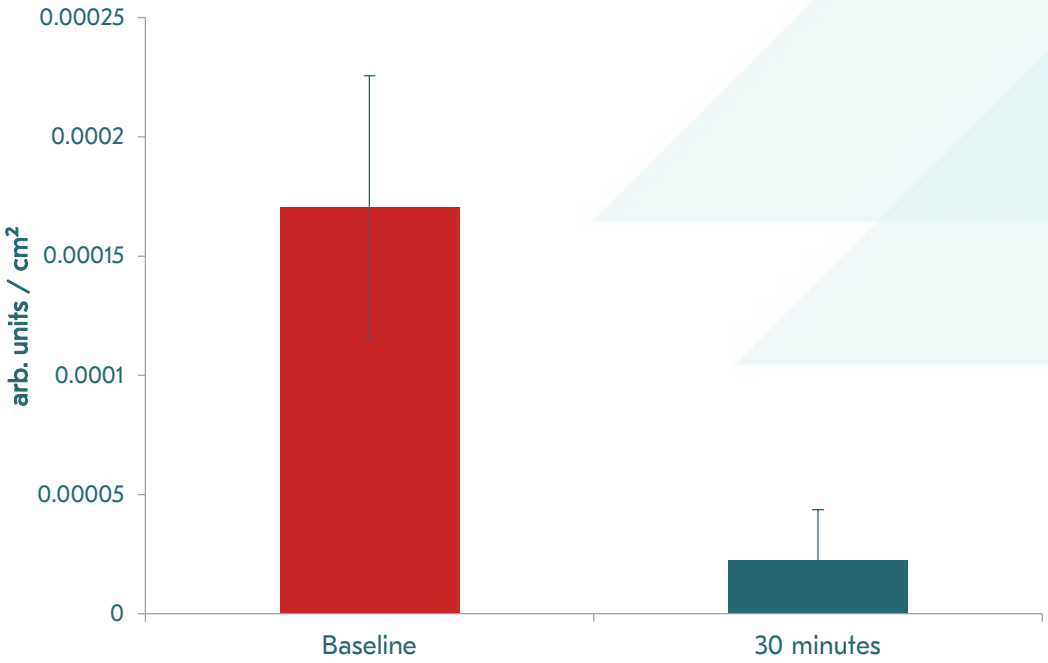
Shampooing on a test area

Head holder

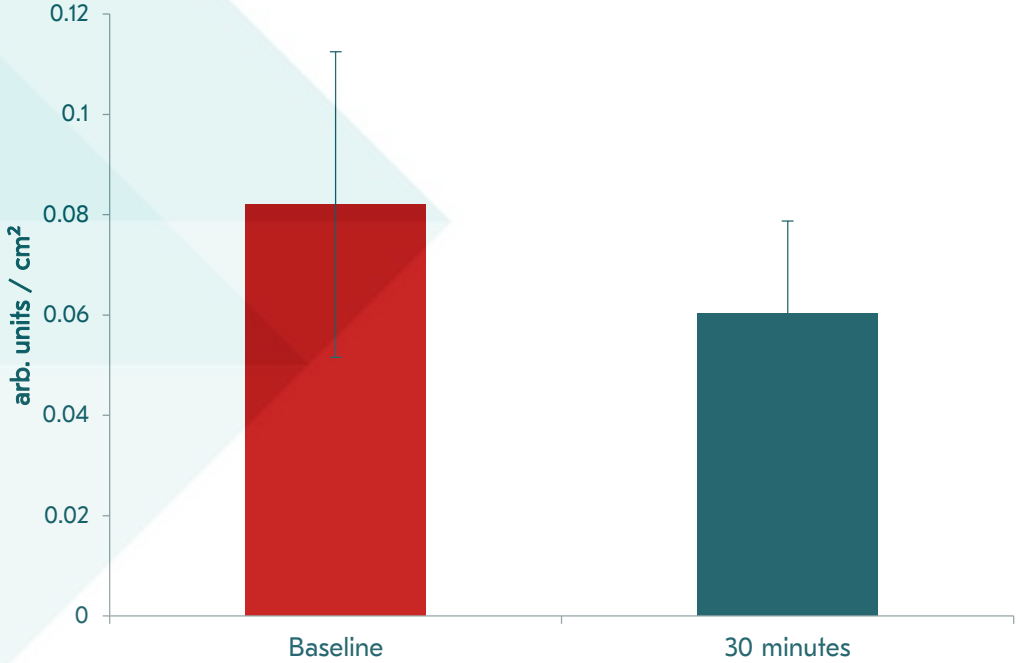
Scalp measurements

Results: Wash out of NMF and Lipids: n = 3; 3 Washes

Washing procedure: 1 ml of 10 % LES shampoo, 1 min washing, 30 sec rinse



Total NMF



Ceramides / Free fatty acids

Measurement at baseline and 30 min after the last washing

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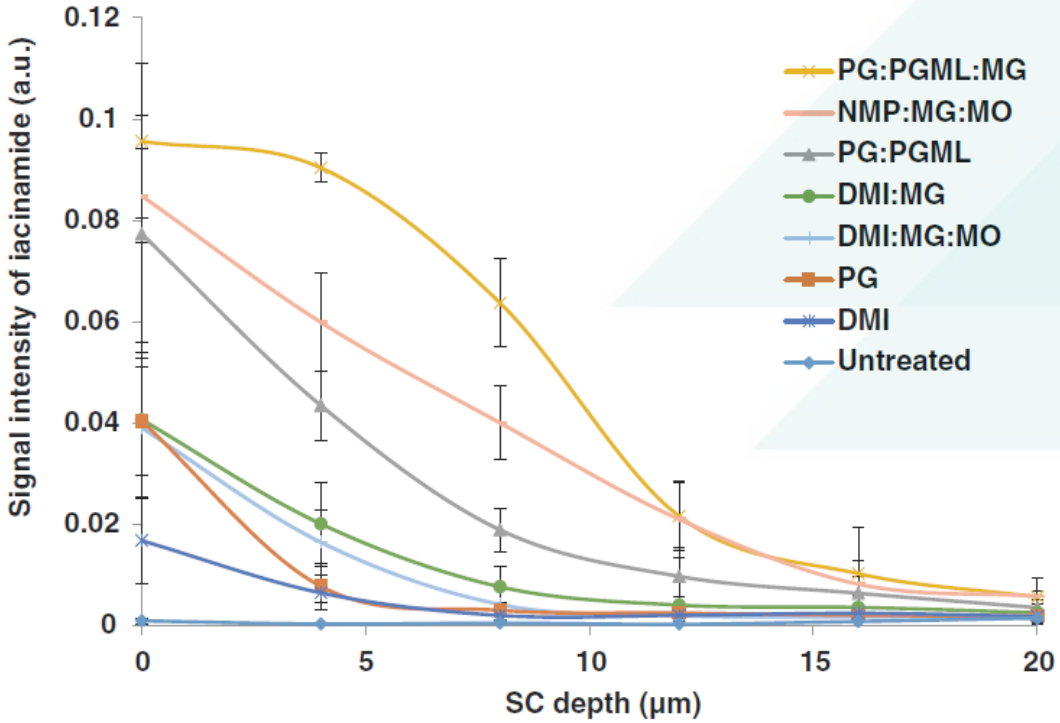
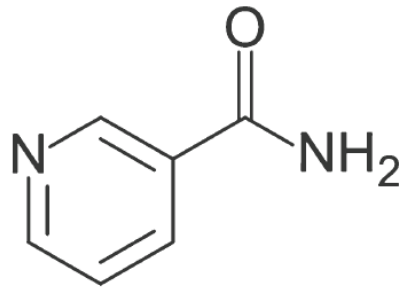
Semi-Quantitative Assessment of Niacinamide, 5%

Different Solvent mixtures; n = 1; 2 mg/cm² for 30 min. on Volar Forearm; 8 repetitions



Intensity of solvent(s) (a.u)

PG = propylene glycol
PGML = Propylene glycole monolaurate
DMI = dimethyl isosorbite
NMP = n-methyl 2-pyrrolidone
MO = mineral oil
MG = Mygliol 812N ®



Niacinamide

Mohammed, D., Matts, P. J., Hadgraft, J., & Lane, M. E. (2014). In vitro—in vivo correlation in skin permeation. *Pharmaceutical research*, *31*(2), 394-400.

Quantitative Measurement of Skin Penetration

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FULL ARTICLE

TRANSLATIONAL BIOPHOTONICS

Method to quantify the in vivo skin penetration of topically applied materials based on confocal Raman spectroscopy

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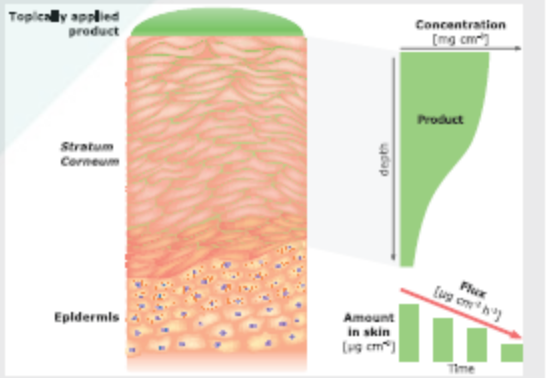
Abstract

This article describes a unique noninvasive capability to determine the concentration (in mg/cm^3) and total amount of topically applied materials in the skin (in µg/cm^2 of skin surface). It is based on in vivo confocal Raman spectroscopy. A theoretical derivation is given of a general method to calcu-

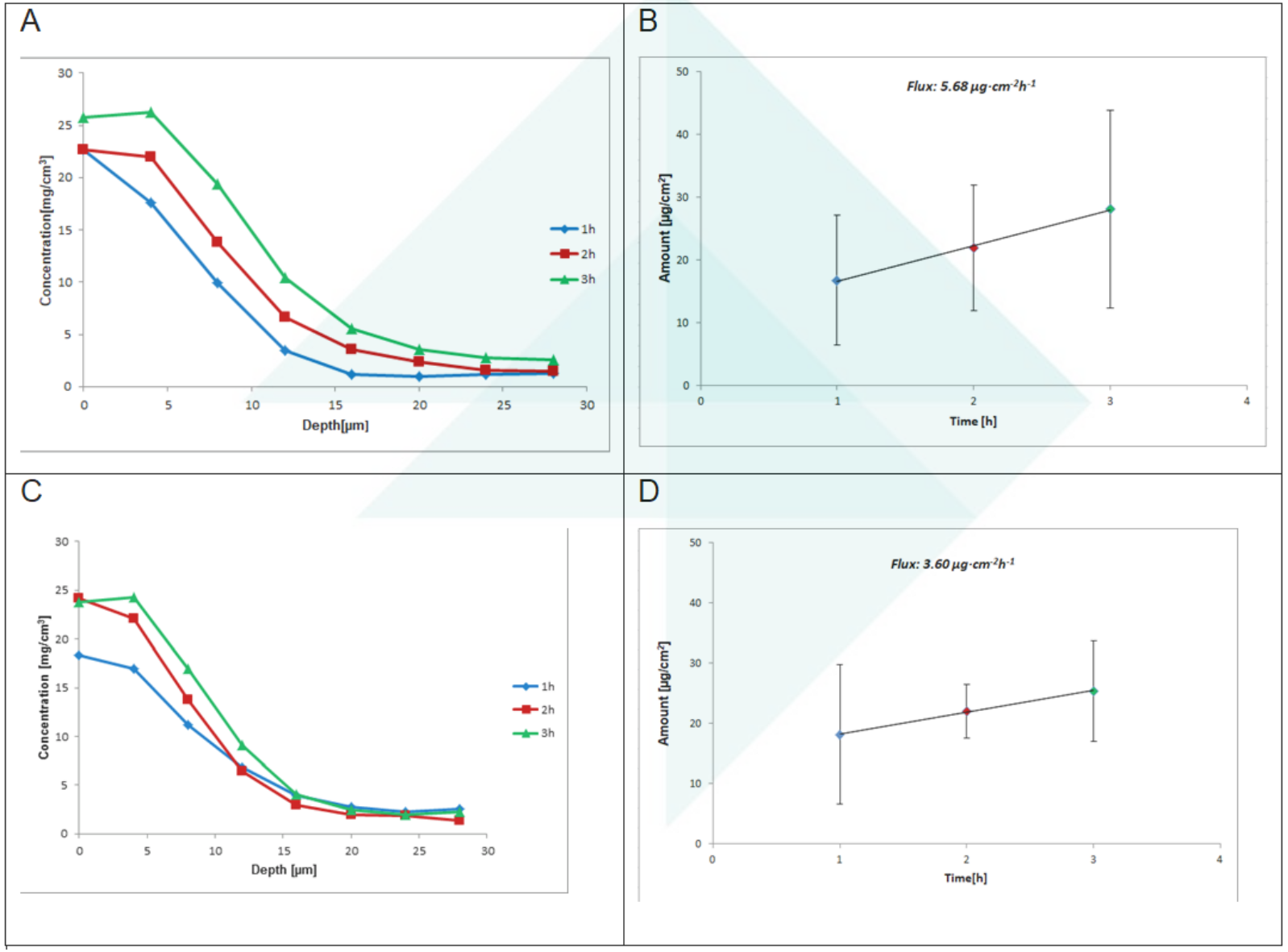


Penetration of Caffeine 2% into Stratum Corneum

Application on 3 areas (volar forearm) for 1, 2 and 3 hours, n = 3

In water

In water + penetration enhancer

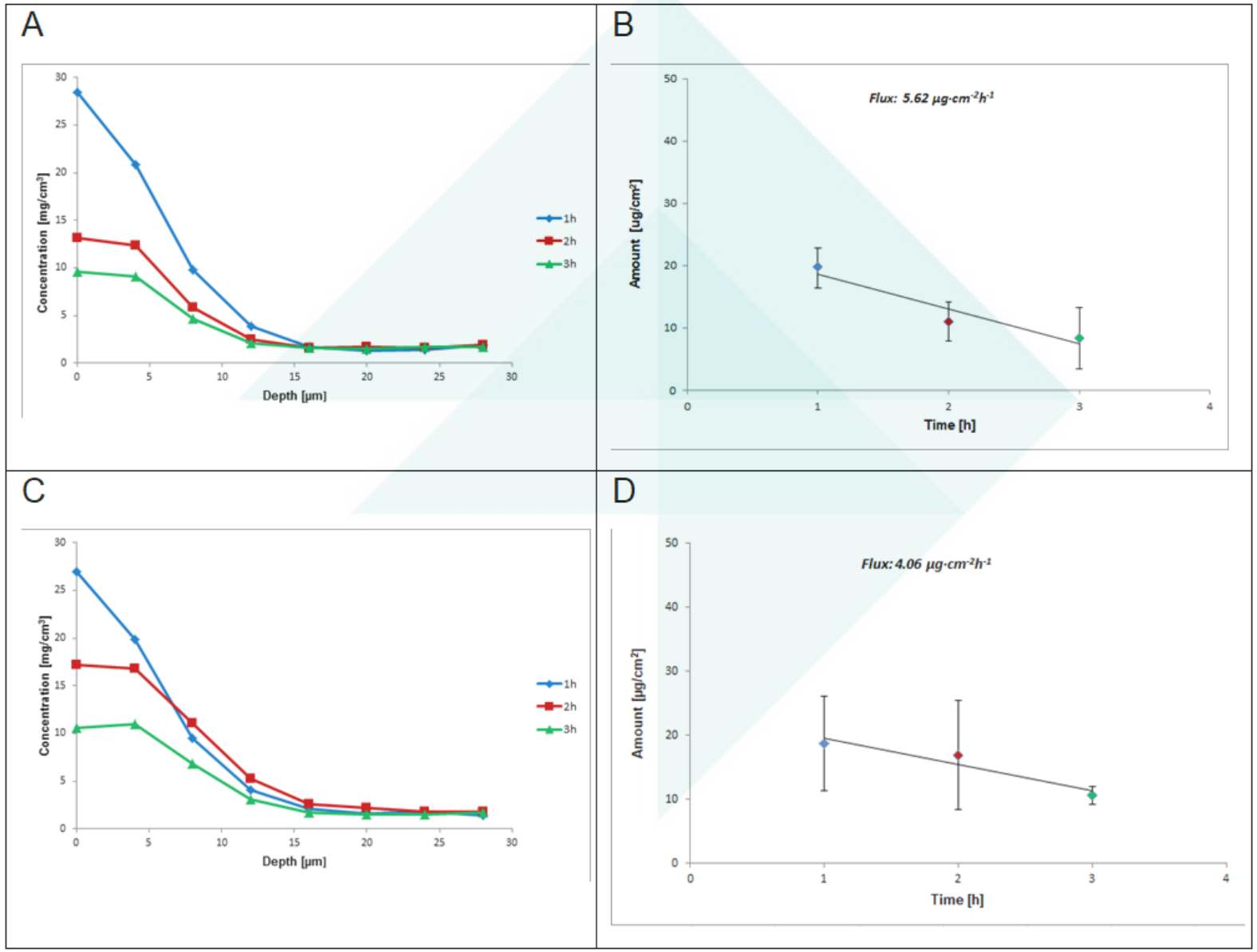


Permeation (Depletion) of Caffeine 2% into Viable Epidermis

Application on 1 area (volar forearm) measured after 1, 2 and 3 hours, n = 3

In water

In water + penetration enhancer



Summary and Conclusions

In vivo Confocal Raman Spectroscopy is a method of many talents

There is a large portfolio of applications that still develop rapidly

Skin moisturization can be assessed based on water profiles across the SC

All main components of SC can be analyzed from the fingerprint spectrum

Specific test locations (Scalp, Axilla, ...) and Subject panels can be measured (children, aged people, ...)

Penetration of molecules into SC and from SC to viable epidermis can be measured quantitatively