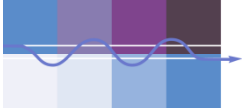


A close-up photograph of a woman with dark hair, eyes closed, splashing water onto her face with both hands. The water is captured in mid-air, creating a dynamic splash. The background is a plain, light color.

IN VIVO SKIN STUDIES BY RAMAN SPECTROSCOPY

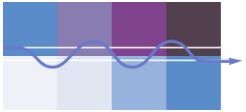
"It works"

Peter Caspers, André van der Pol
Johanna de Sterke, Paula de Galan
River Diagnostics B.V.
The Netherlands



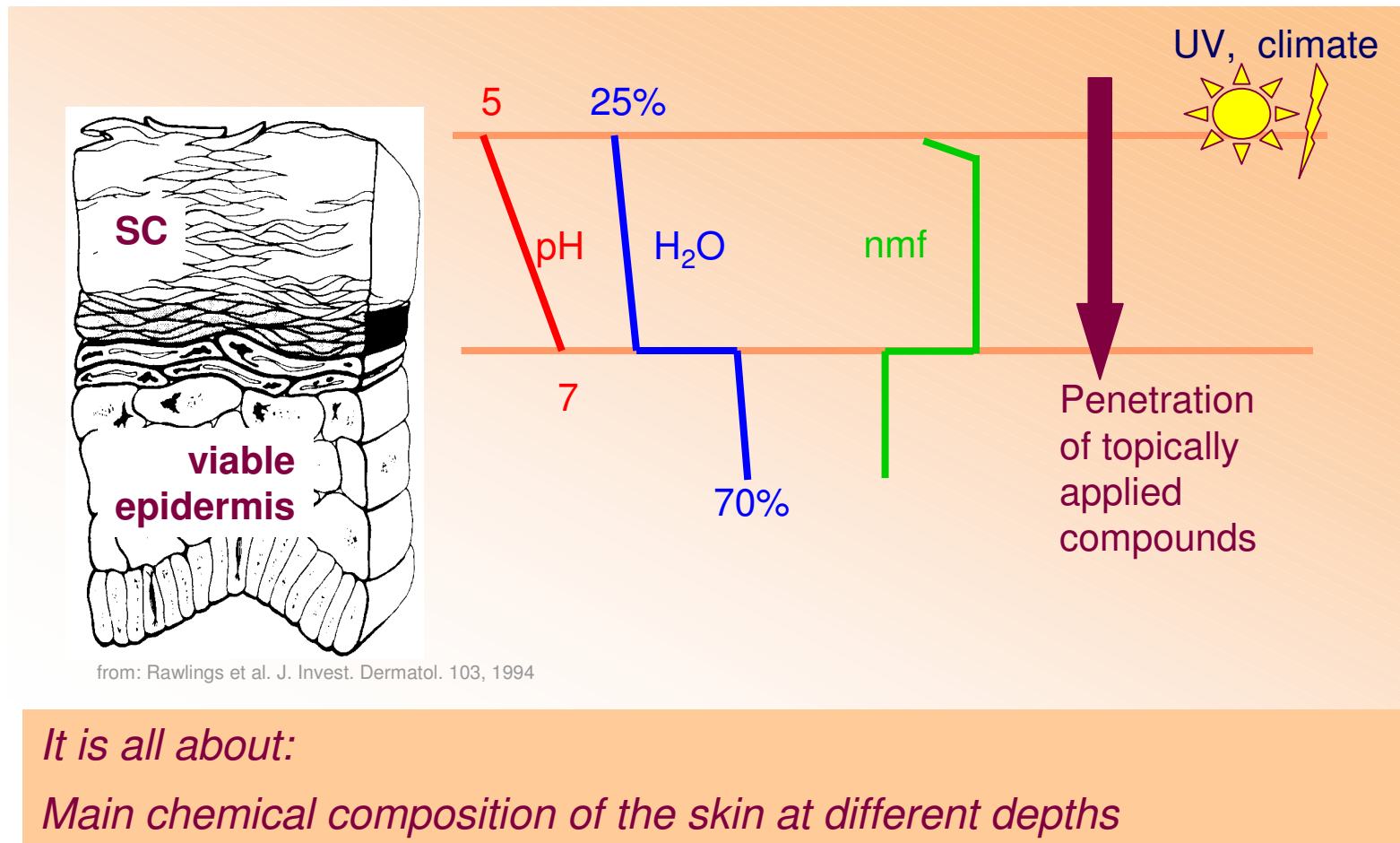
- Information in Raman spectra of skin
- How to extract practical information
- Applications & validation

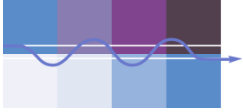




Information in Raman spectra of skin

3

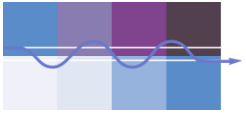




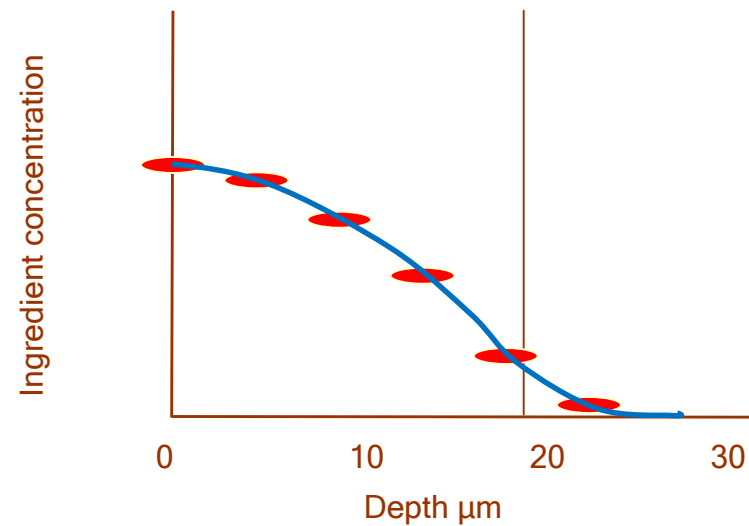
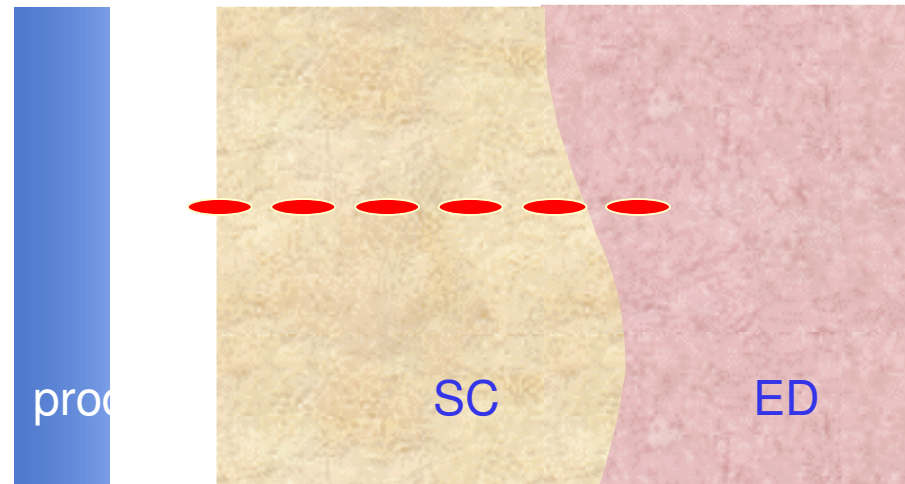
Information in Raman spectra of skin, examples

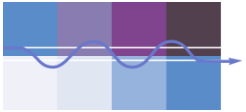
- skin characterisation
 - water, nmf, lipids, carotenoids
 - typing, aging effects, ethnic effect, cultural effects
 - neutraceutical
- Penetration and penetration kinetics
 - glycerol, dmso, lactate, ethanol, caffeine, menthol, nicotinamide, uv absorbers, oils, retinol, carotenes
 - formulation optimization
 - transdermal drug delivery, various pain relief agents
 - Toxicological: pyrene, toluene, butoxyethanol
- metabolic processes
 - hydrolysis of esters
- effects of products
 - moisturization
 - pH modulation
- effects of environment / skin treatment
 - humidity, season, climate, solar exposure
 - bathing, washing
- medical (diseased/disfunctional skin)
 - Atopic dermatitis



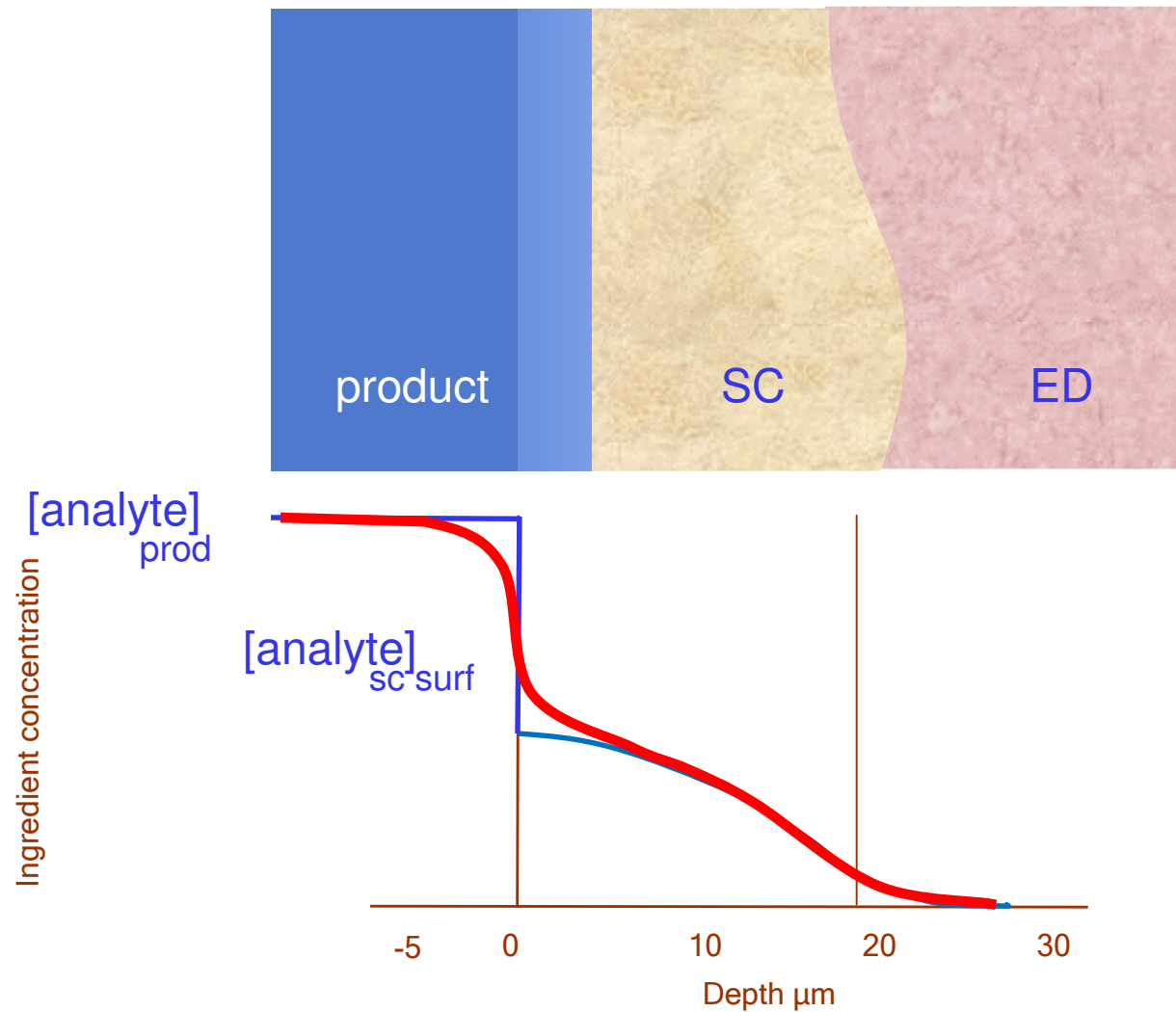


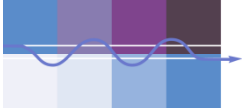
How to extract practical information, concentration profiles



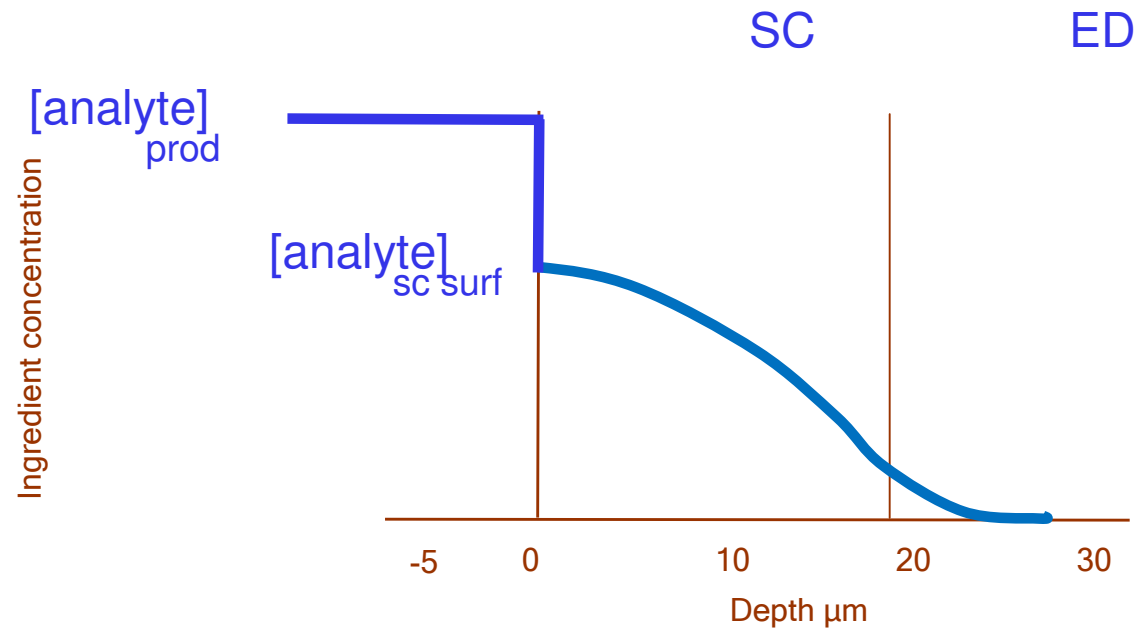


How to extract practical information, partition coefficients



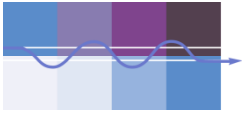


How to extract practical information, partition coefficients



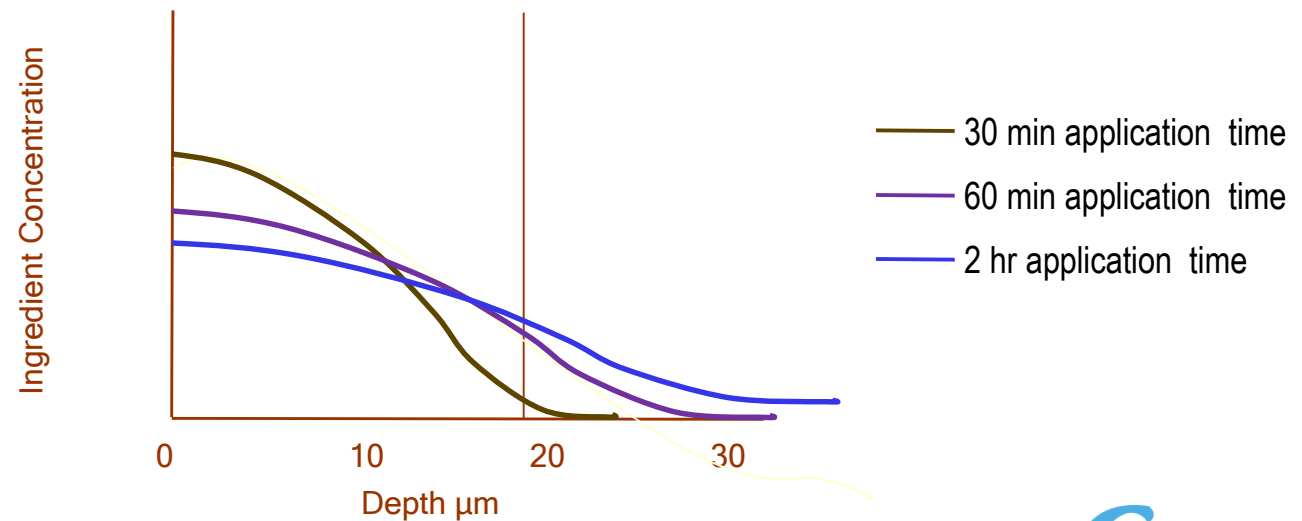
$$\text{partition coefficient} = K = \frac{[\text{analyte}]_{\text{SC}}}{[\text{analyte}]_{\text{prod}}}$$

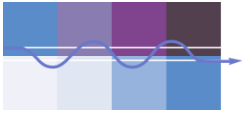




How to extract practical information, kinetics of diffusion

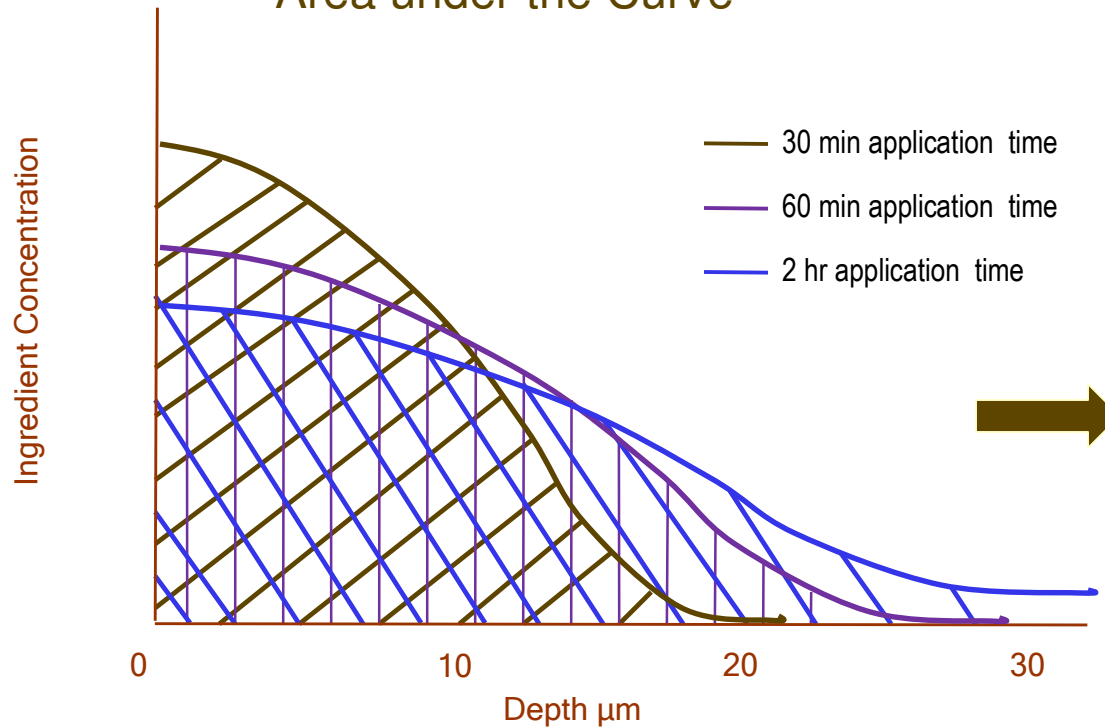
8



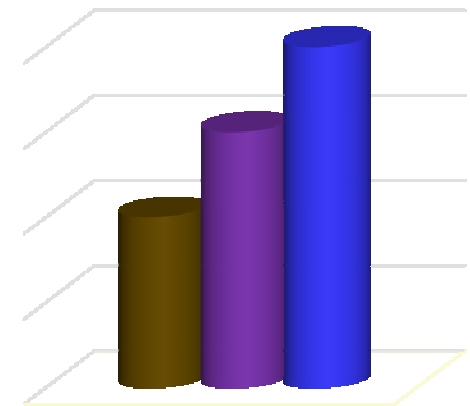


How to extract practical information, absorption and flux calculations

Area under the Curve



Absorbed volume product

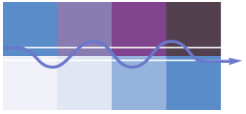


Ingredient Concentration in mmol/gr keratin



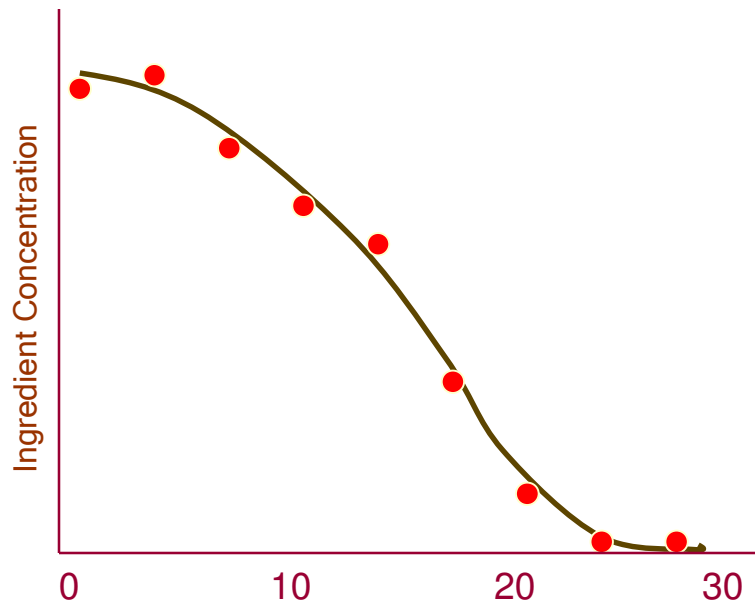
Absorbed amount in $\mu\text{g}/\text{cm}^2$





How to extract practical information, diffusion kinetics, Fick's 2nd law

Analysis of shape of the
Curve



Theoretical:

$$\frac{d([analyte])}{dt} = D \frac{d^2([analyte])}{dz^2}$$

Simple solution 1:

$$[analyte](z,t) = [analyte]_{t=0} \cdot \operatorname{erf}\left(\frac{z}{\sqrt{4Dt}}\right)$$

Simple solution 2:

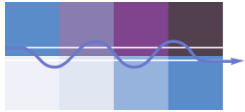
$$[analyte](z,t) = \frac{[analyte]_{t=0}}{A\sqrt{\pi Dt}} e^{-\frac{z^2}{4Dt}}$$

analyte concentration in mmol/gr keratin



Diffusion Coefficient D



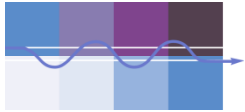


applications - caffeine diffusion constants

Published	Caffeine Application	D (cm ² /s)	Skin type
Southwell and Barry, JID 1983	1 %, water ¹⁴ C labels	4.5·10 ⁻¹⁰	Human in vitro
Nalenz, Dissertation, Uni Basel, 2006	?	1.7·10 ⁻¹⁰	Human in vitro
Hansen et al., Eur J Pharmac. And Biopharmac, 2008	1.25 %, water	3.9·10 ⁻¹¹ D was found application time dependent)	Human in vitro
River Diagnostics, 2009	1.8 %, water	8.28·10 ⁻¹²	Human in vivo

- D is very likely show large inter-individual variation
- D is a complicated parameter, as shown by the time dependence of D (by Hansen)
- Data on sampled skin all show higher, and sometimes much higher, D values. Excision of skin likely induces artefacts that influence the skin barrier as compared to in vivo skin.
- Best agreement found with the newest data.

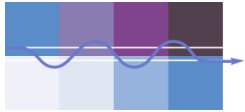




applications – caffeine fluxes and absorption

Published	Caffeine Application	Fluxes/ Transfer	Skin type
Southwell and Barry, JID 1983	1%, water ¹⁴ C labels	1.3 µg/cm ² /h	Human in vitro
Dias et al. 1999	3% in commercial formulation	15 - 150 µg/cm ²	Human in vitro
Pugh et al. 2004	4 µg/cm ² in ethanol	0.48±0.16 µg/cm ²	Human in vitro
Van de Sandt et al. 2004		2.24±1.43 µg/cm ² /h	Human in vitro
Potard et al. 1999	3% in commercial formulation	2.3±2.0 µg/cm ²	Human in vitro
Nicoli et al. 2004	330 µg/cm ²	1 - 100 µg/cm ²	Rabbit ear in vitro
Hansen et al. 2008	1.25 % water	25.6 µg/cm /h	Human in vitro
River Diagnostics	1.8% in water (3.5 mg/cm ²)	25 µg/cm ² /h	Human in vivo





Pudney et al., Applied Spectroscopy 61(8): 2007

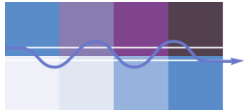
An *In Vivo* Confocal Raman Study of the Delivery of *Trans*-Retinol to the Skin

PAUL D. A. PUDNEY,* MICKAËL MÉLOT, PETER J. CASPERS, ANDRE VAN DER POL, and GERWIN J. PUPPELS

Measurement Science Unit, Unilever R&D, Colworth Science Park, Sharnbrook, Bedford, MK44 1LQ (P.D.A.P., M.M.); and River Diagnostics B.V., Rotterdam, The Netherlands (P.J.C., A.v.D.P., G.J.P.)

- **Solution 1:** 70 % ethanol, 30 % PG and 0.3 % *trans*-retinol
- **Solution 2:** 99.7 % MYRITOL®318 and 0.3 % *trans*-retinol
- monitor penetration for 0-6 hrs after application

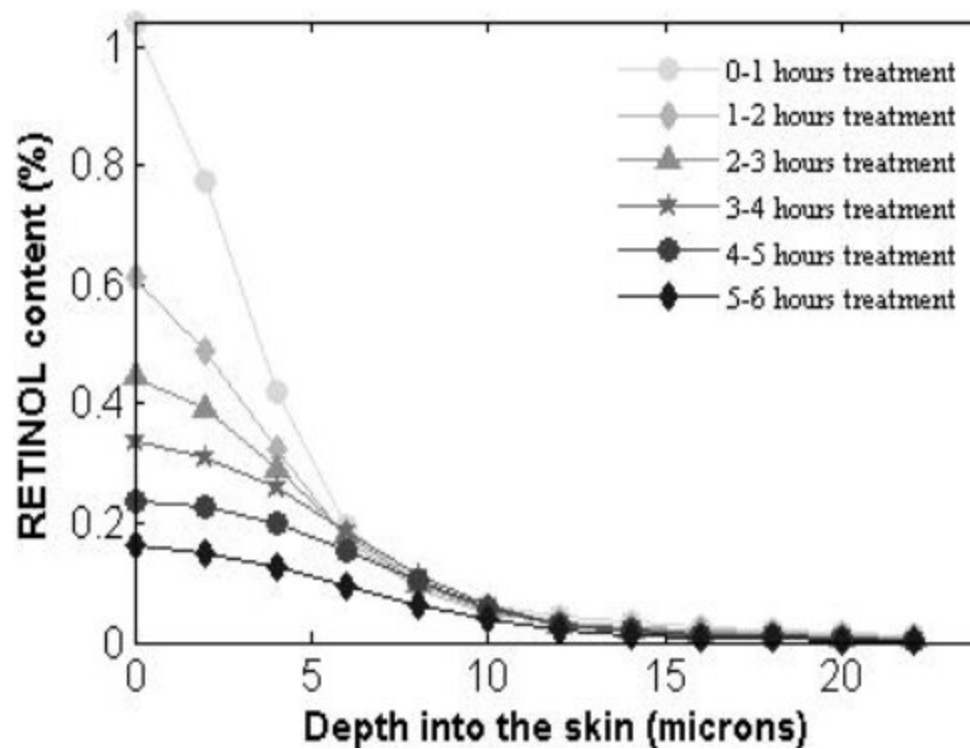


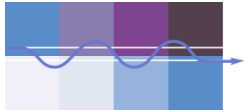


applications - retinol delivery

14

Pudney et al., Applied Spectroscopy 61(8): 2007



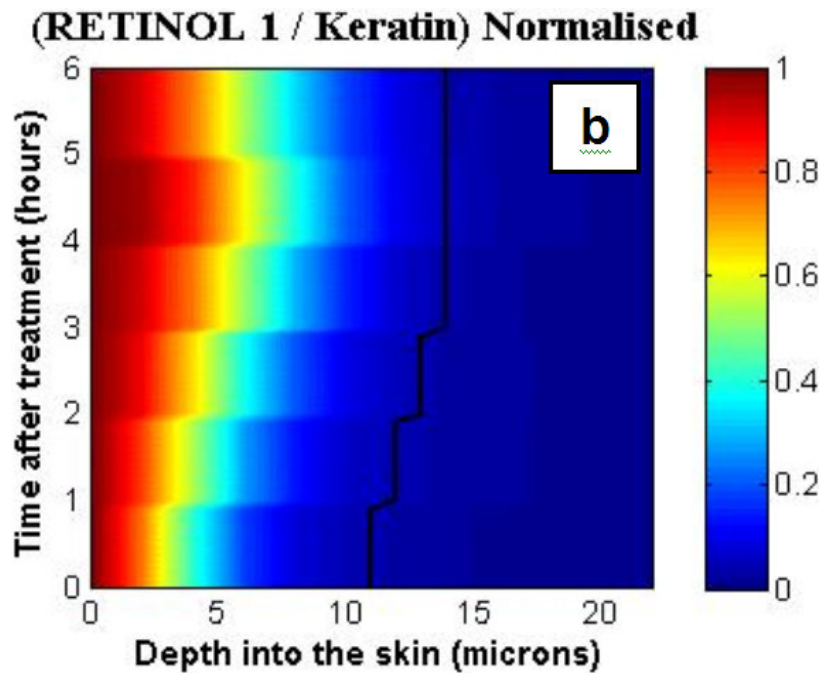


applications - retinol delivery

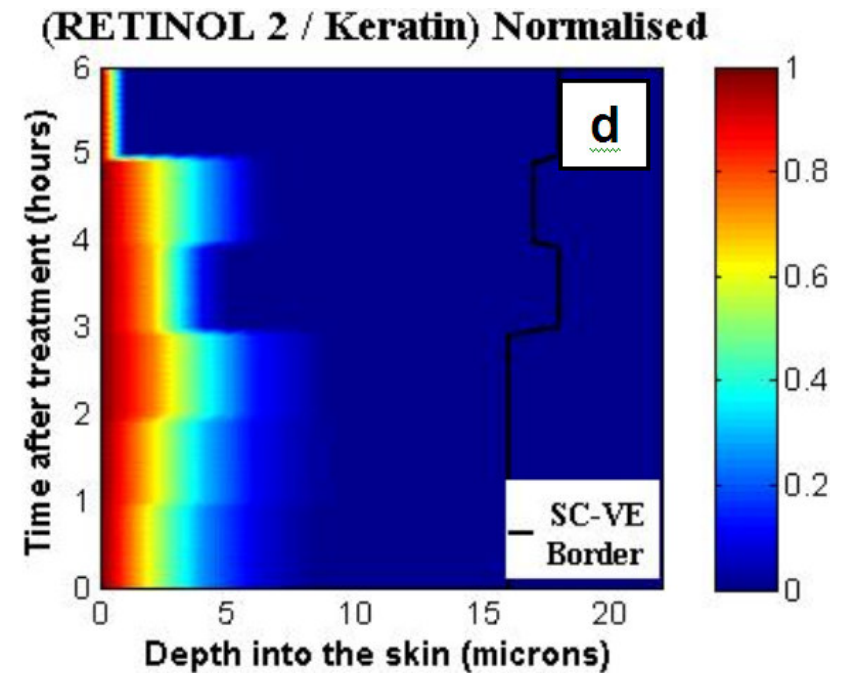
15

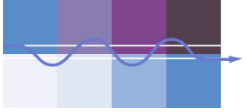
Pudney et al., Applied Spectroscopy 61(8): 2007

in PG/EtOH



in MYRITOL[®]318





validation – skin hydration

16

- J. Wu and T. Polefka, Colgate-Palmolive, International Journal of Cosmetic Science, 30, 47–56, 2008
- Analysis of total water content of moisturized pigskin stratum corneum by Raman and independently by Karl Fischer titration



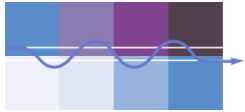
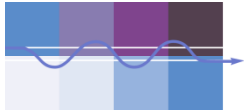


Table II Water content of the stratum corneum determined by Karl Fischer method and confocal Raman microspectroscopy

Relative humidity (%)	Karl Fischer		Confocal Raman	
	Water content (% mass)	SD	Water content (% mass)	SD
0	7.7	0.80	8.0	1.52
11	8.9	0.75	10.2	0.98
32	11.0	0.91	10.7	0.74
75	13.8	0.98	15.5	0.77
100	24.4	0.94	25.9	0.34

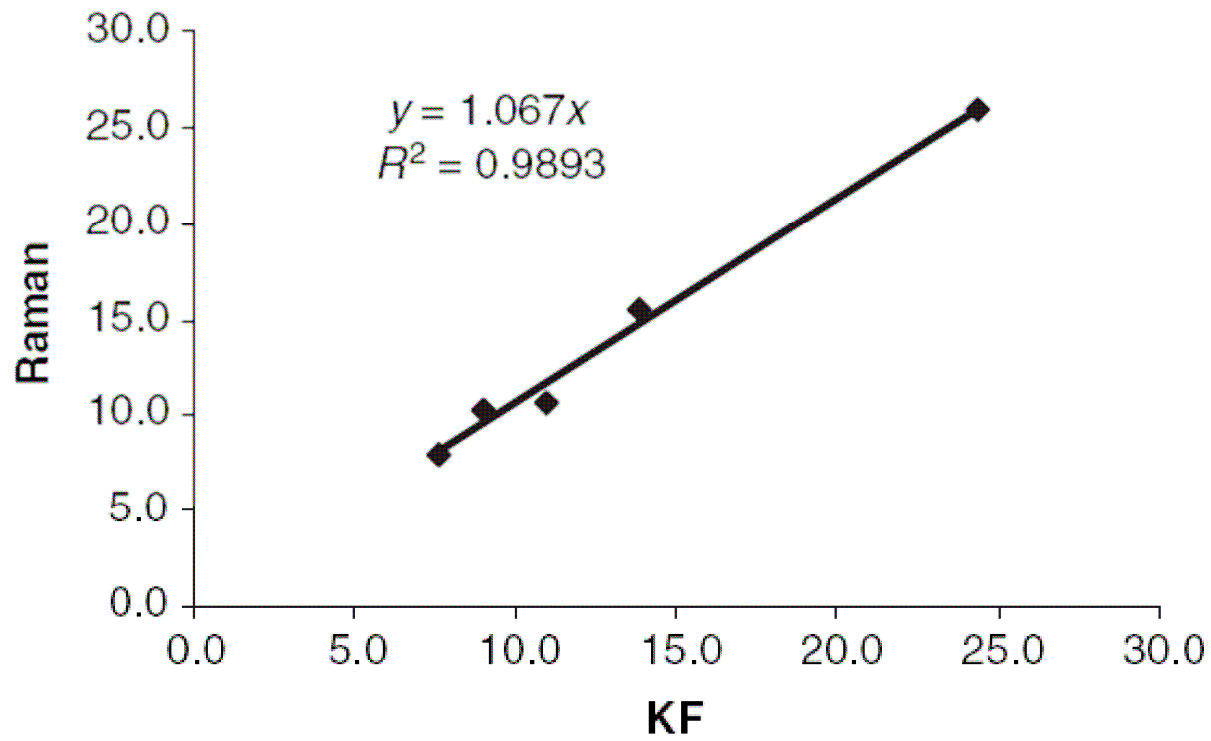
SD, standard deviation. *n* = 3 for each relative humidity condition, the total number of samples was 15.

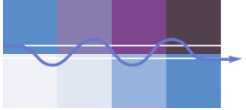




skin hydration: validation using pigskin and KF titration

18

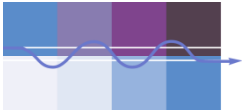




skin hydration: swelling

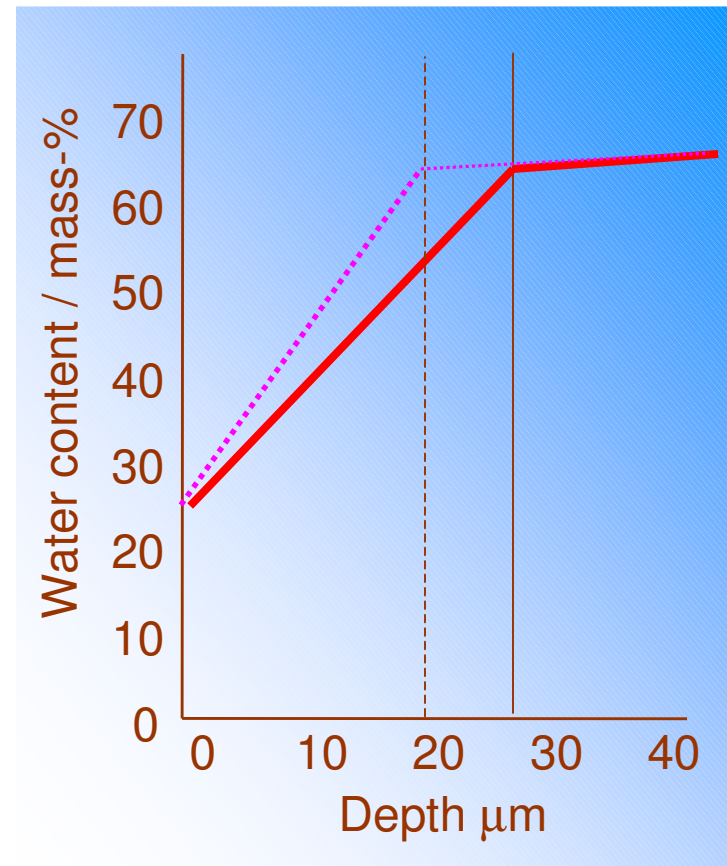
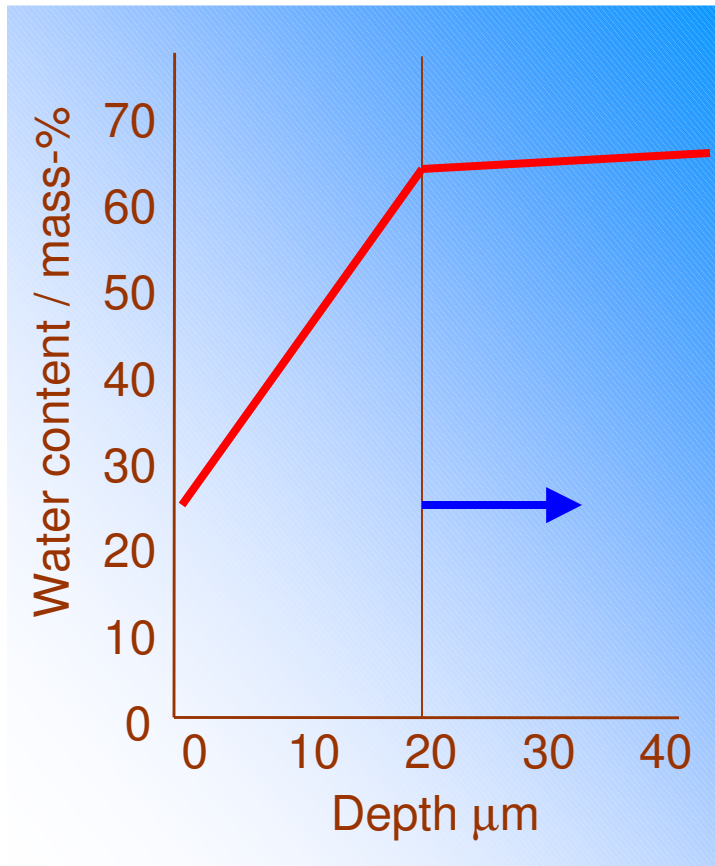
19

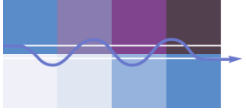




skin hydration: swelling

20

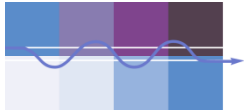




The predicted swellings are indeed found with other methods:

- Good correlation between thicknesses and their changes upon moisturization as measured by confocal Raman and Optical Coherence Tomography (OCT)
- No direct correlation between capacitance readings and Raman results, from the paper by:
 - Measuring the effects of topical moisturizers on changes in stratum corneum thickness, water gradients and hydration in vivo J.M. Crowther, A. Sieg, P. Blenkiron, C. Marcott, P.J. Matts, J.R. Kaczvinsky and A.V. Rawlings, Br. J. Dermatol. 2008 159 567-577.
- Good correlation for thickness also for CVM (S. Bielfeldt et al., in preparation).





applications - skin hydration, syndet-bar vs. soap-bar

22

Wu et al., J Cosm Sci 30: 47–56 (2008)

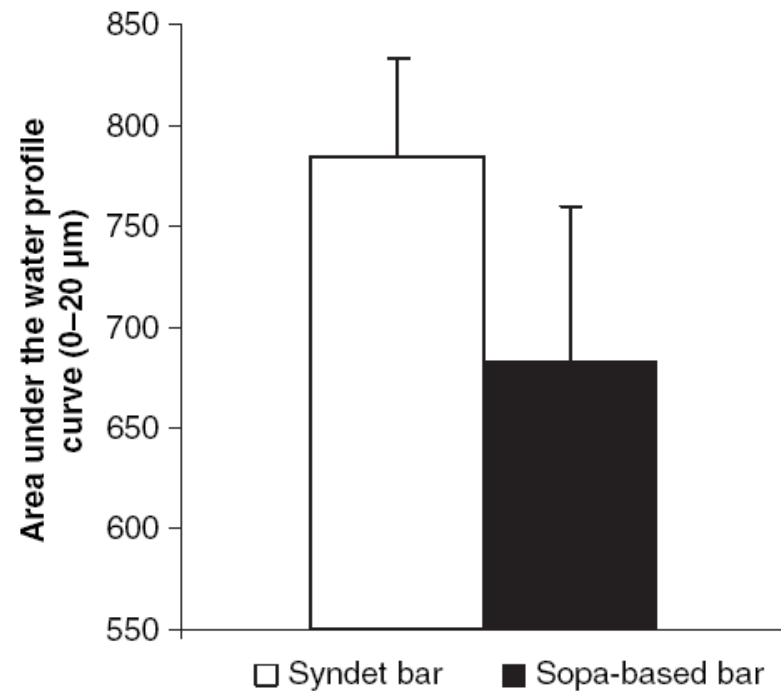
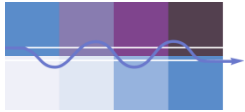
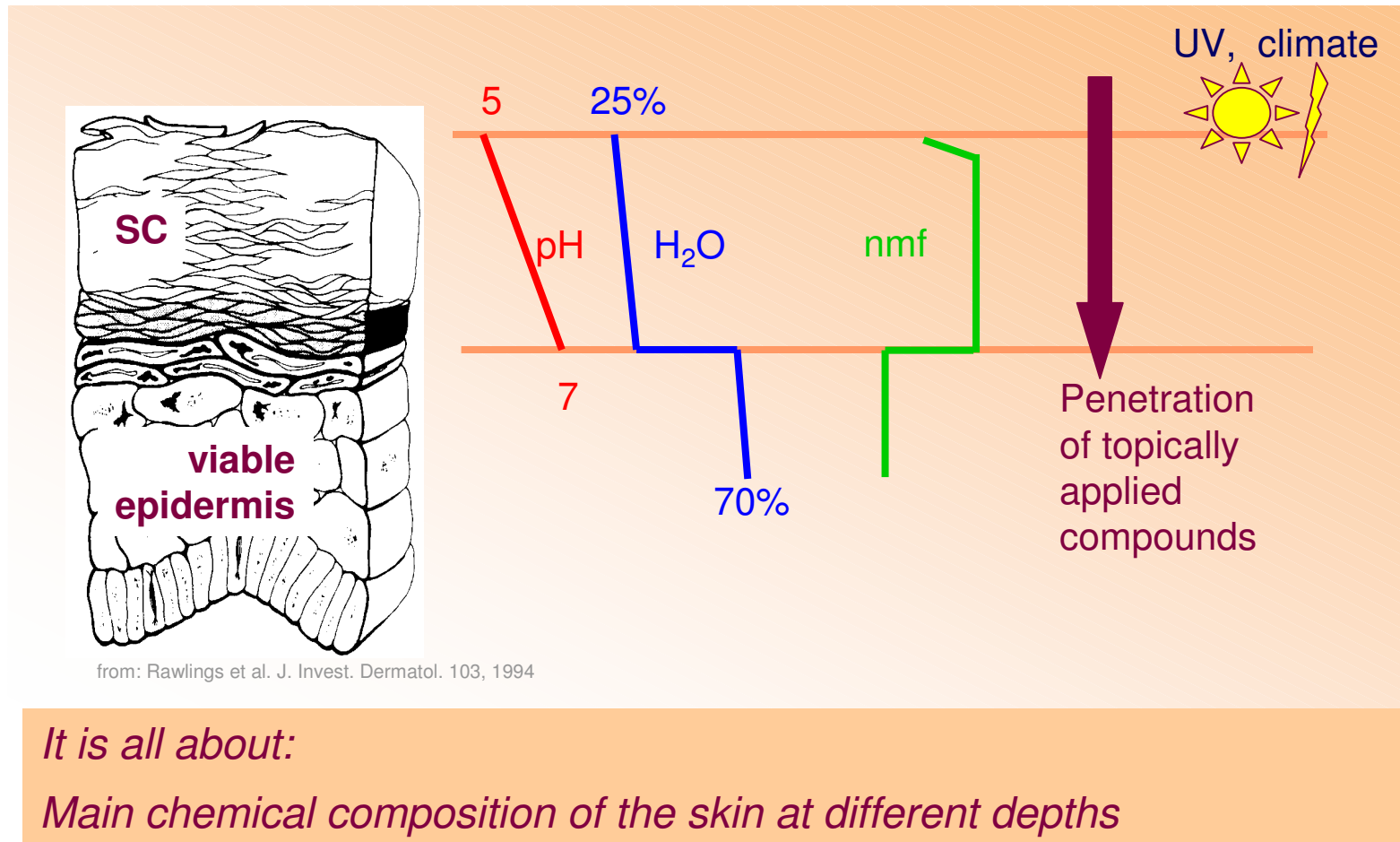


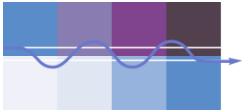
Figure 7 Area under the water profile curve integrated over 0–20 μm for syndet and soap-based bar. $n = 6$. The total number of profiles from each product was 30.





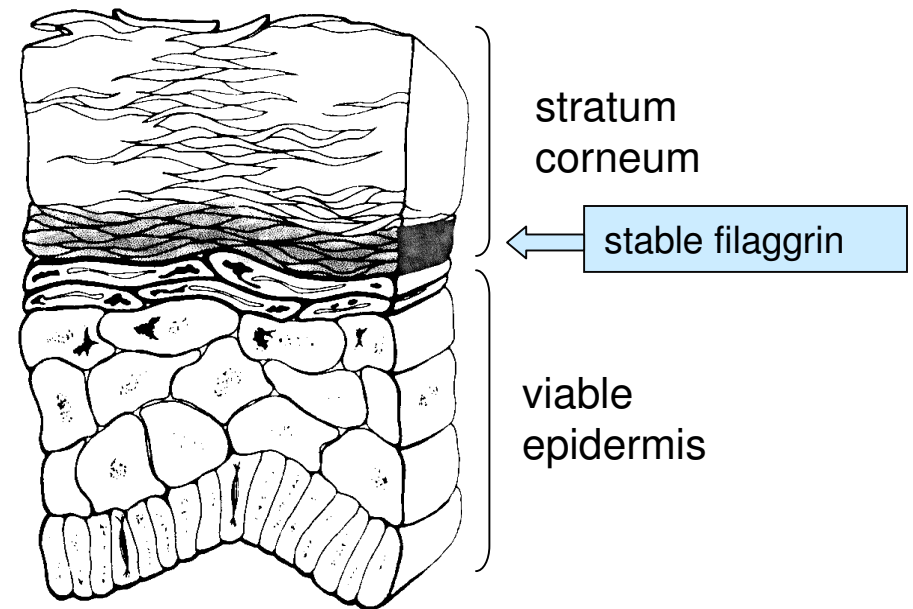
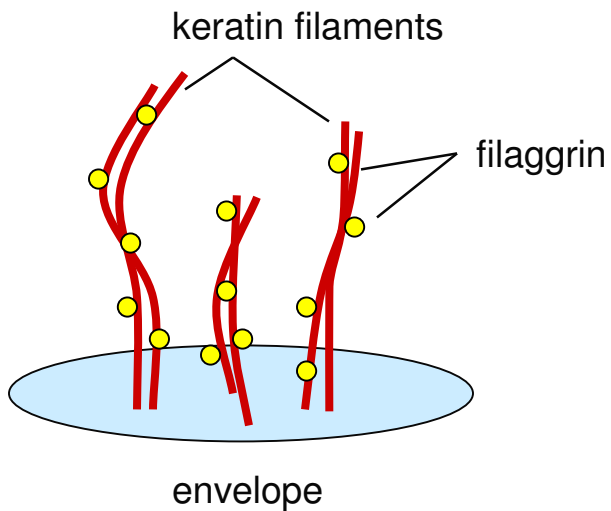
information in Raman spectra of skin





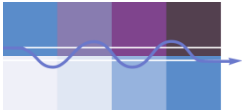
Filaggrin and atopic dermatitis (eczema)

organization of the keratin cytoskeleton



Drawing from A.V.Rawlings et al., JID 103 (1994)

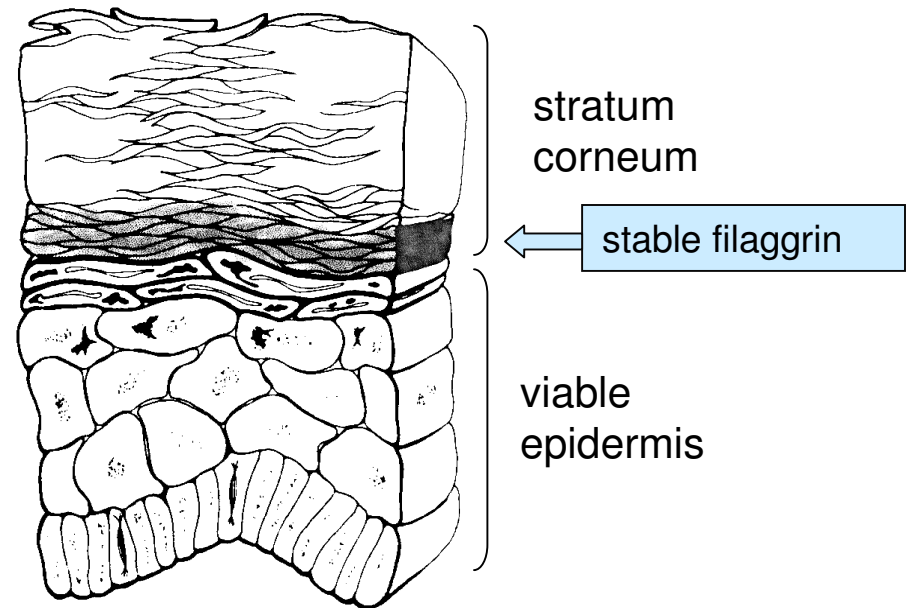
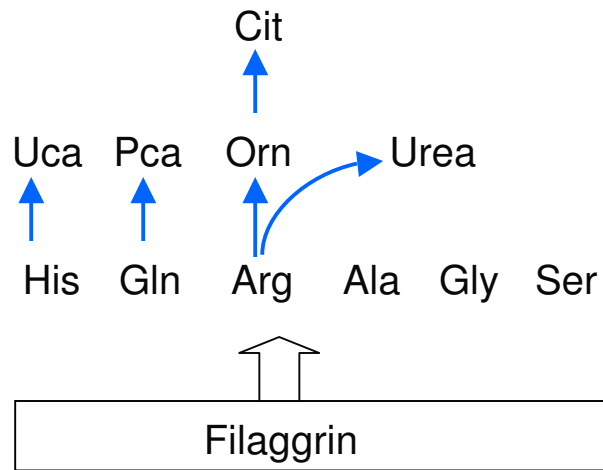




Filaggrin and atopic dermatitis (eczema)

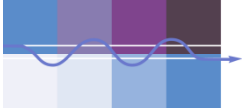
25

source for the NMF



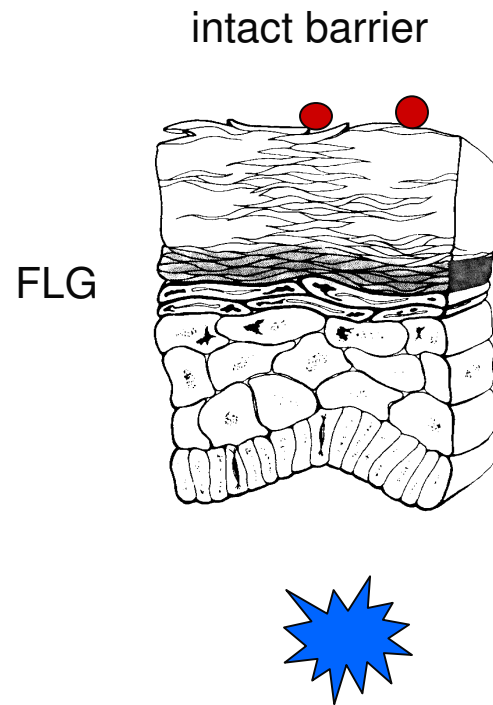
Drawing from A.V.Rawlings et al., JID 103 (1994)

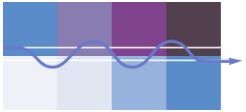




sensitization and skin barrier

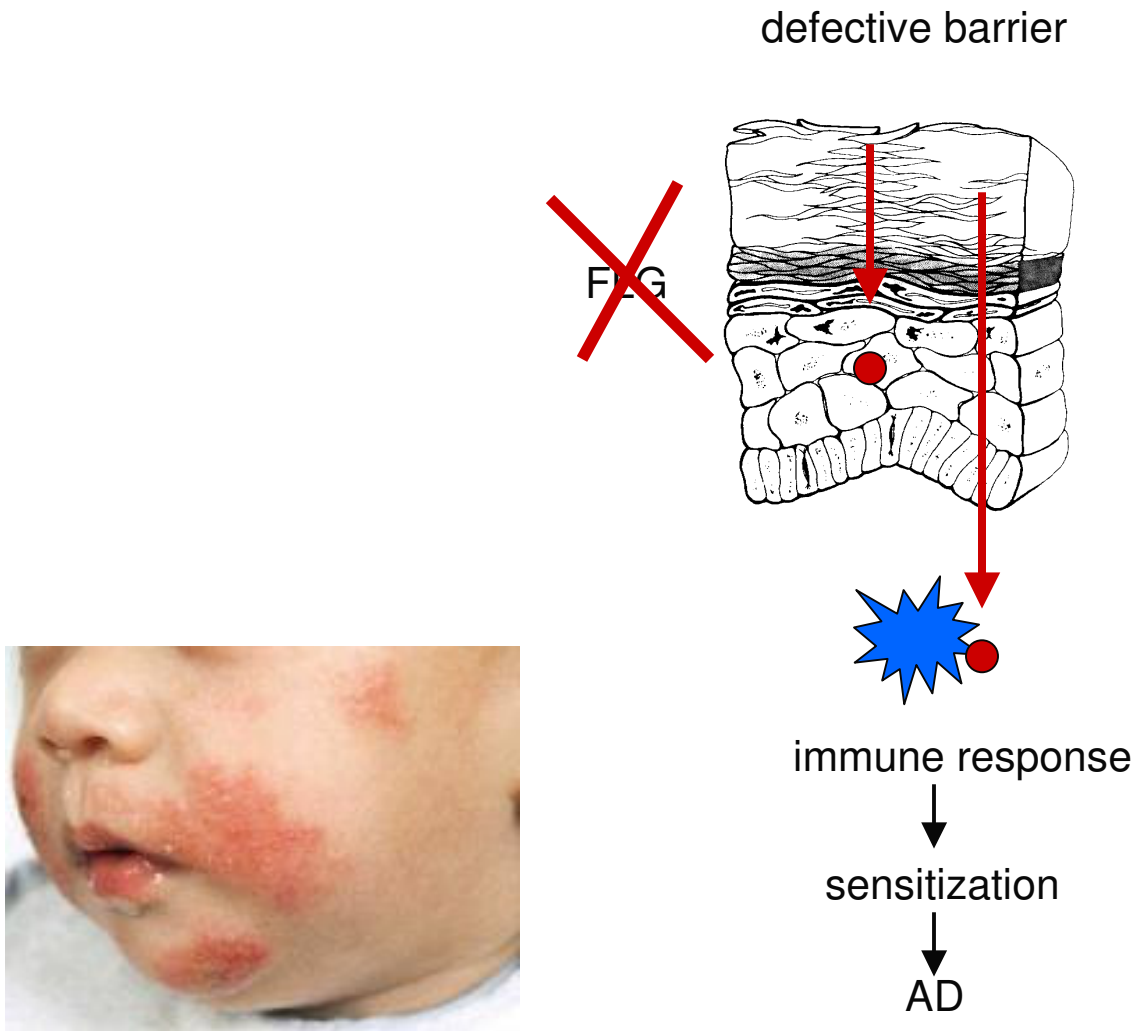
26

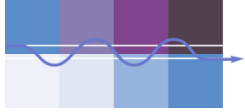




sensitization and skin barrier

27





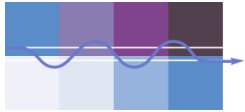
Loss-of-function mutations in the filaggrin gene lead to reduced levels of natural moisturizing factor in the stratum corneum

Sanja Kezic^{1,8}, Patrick M. J. H. Kemperman^{2,8}, Ellen Koster¹, Cindy de Jongh¹, Bing H. Thio², Linda E. Campbell³, Alan D. Irvine^{4,5} and W. H. Irwin McLean³, Gerwin J. Puppels^{6,7} and Peter J. Caspers^{6,7}

J Invest Dermatol 128:2117-19 (2008)

- 149 volunteers + 10 AD patients
- screened for R501x, 2282del4, R2447x, S3247x
- included in study: 16 carriers and 23 wild-types
- in vivo Raman assessment of nmf (on forearm and thenar)

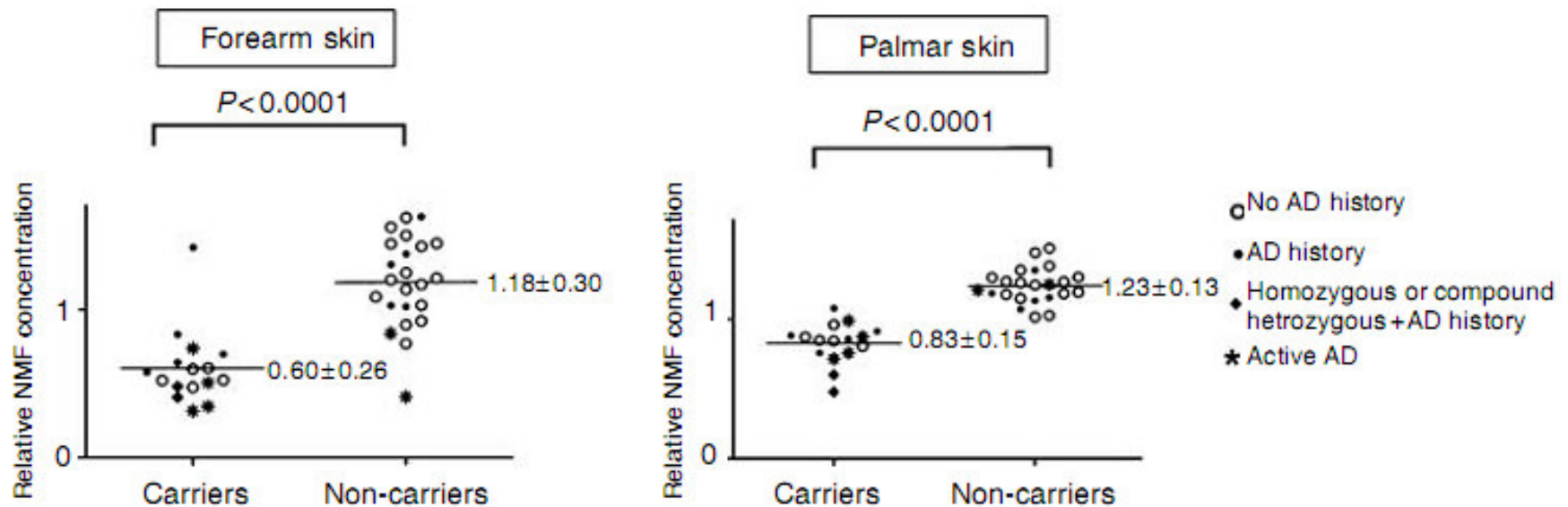


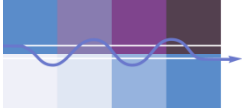


filaggrin - Raman study

29

Kezic et al., *J Invest Dermatol* 128:2117-19 (2008)





Filaggrin and atopic dermatitis (eczema)

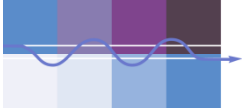
30



- early diagnosis & intervention
- more differentiated diagnosis
- more targeted intervention

...happy babies





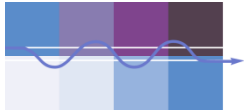
Conclusions

31

Raman spectroscopy on skin

- validated in several areas
- more and more routinely used
- *It works!*





confocal Raman microspectroscopy

32

