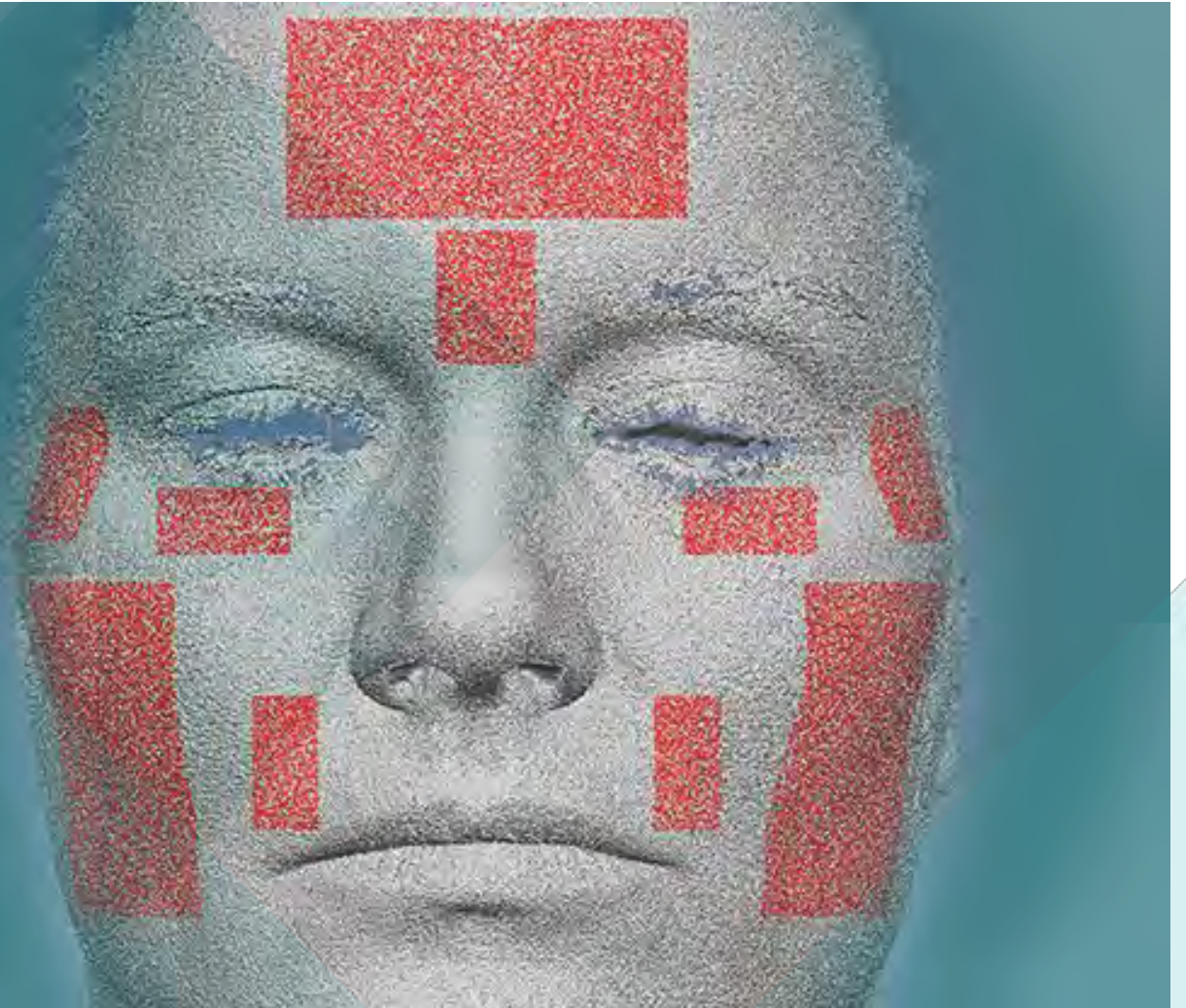


# How to substantiate Anti-Aging Claims?

Gunja Springmann





# How to substantiate Anti-Aging Claims?

## Signs of aging and ways to measure product efficacy

- Intrinsic and extrinsic aging
- Clinical parameters
- Testing methodologies for the in-vivo investigation such as (collagen, moisture, texture, redness, pigmentation, pores, wrinkles)
- Extrinsic Stressors such as pollution, UVR, blue light
- Biomarkers



# Intrinsic Aging



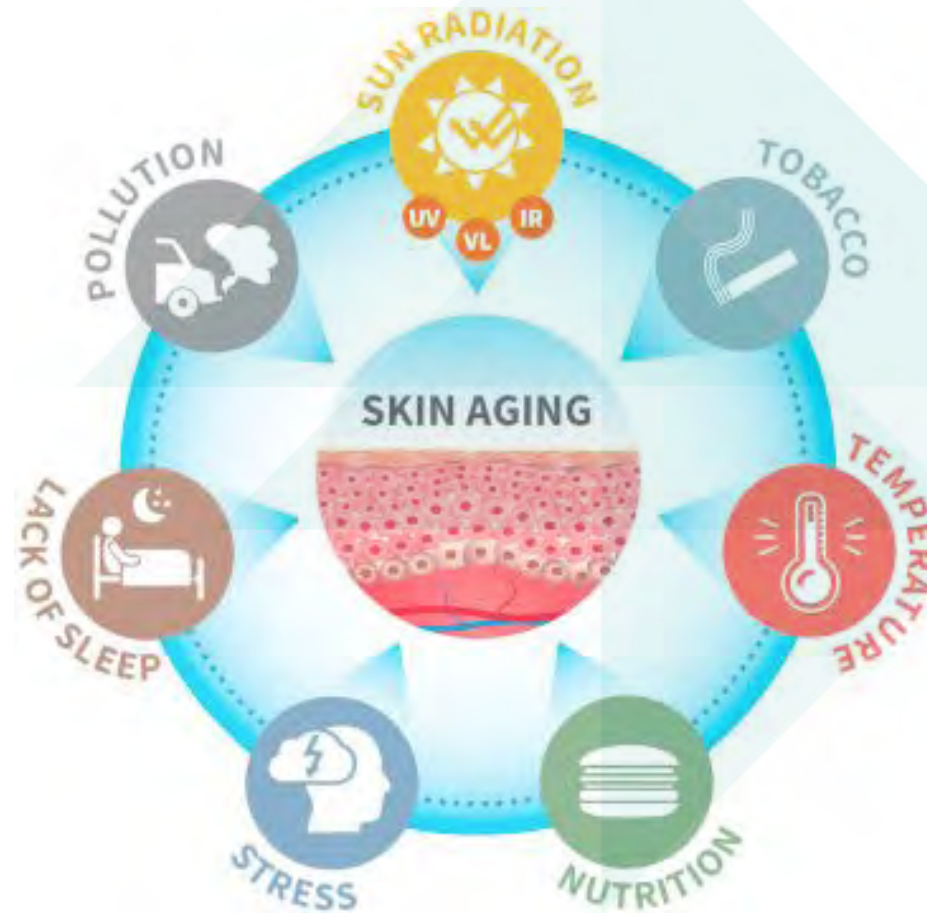
Time



Genetics



# Extrinsic Aging



The skin aging  
exposome  
Jean Krutmann, M.D.;  
Journal of  
Dermatological  
Science



# Agenda

- Intrinsic and extrinsic aging
- Clinical parameters
- Testing methodologies for the in-vivo investigation explained
- Results explained





# Clinical Assessment



## Example Areas

- Crow's Feet
- Forehead
- Glabellar
- Interocular
- Under Eye
- Preauricular
- Perioral Area
- Upper lip wrinkles
- Marionette Lines
- Nasolabial Fold
- Cheek Folds

## Signs of Aging

- Sagging
- Eyelid Sagging
- Lifting
- Firmness
- Acne
- Lesion counts
- Pores
- Dryness
- Eye Bags
- Dark Circles
- Photodamage



# Objective assessments by trained graders

- Assessors: physicians, beauticians, hair dressers or other trained experts
- Defined and trained scoring system which is robust and reproducible
- Before and after application assessments or comparison to reference possible
- Reproducible and sensitive, similar to instrumental assessments
- Support findings of instrumental or biochemical studies

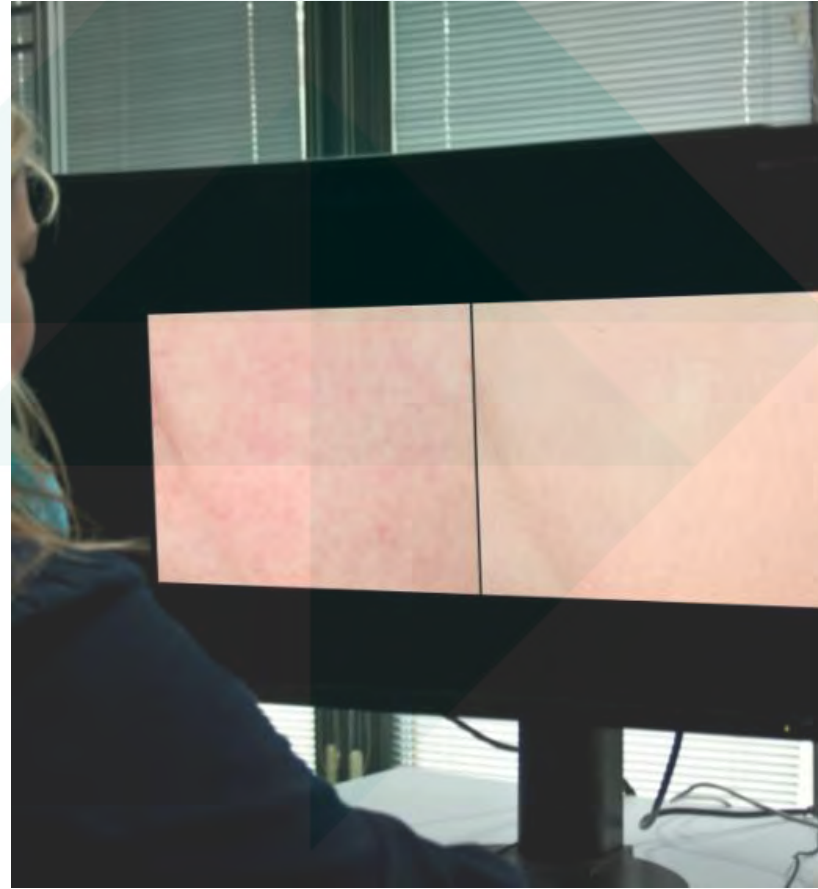




# Presentation of Images for Expert Rating



Color-calibrated monitor



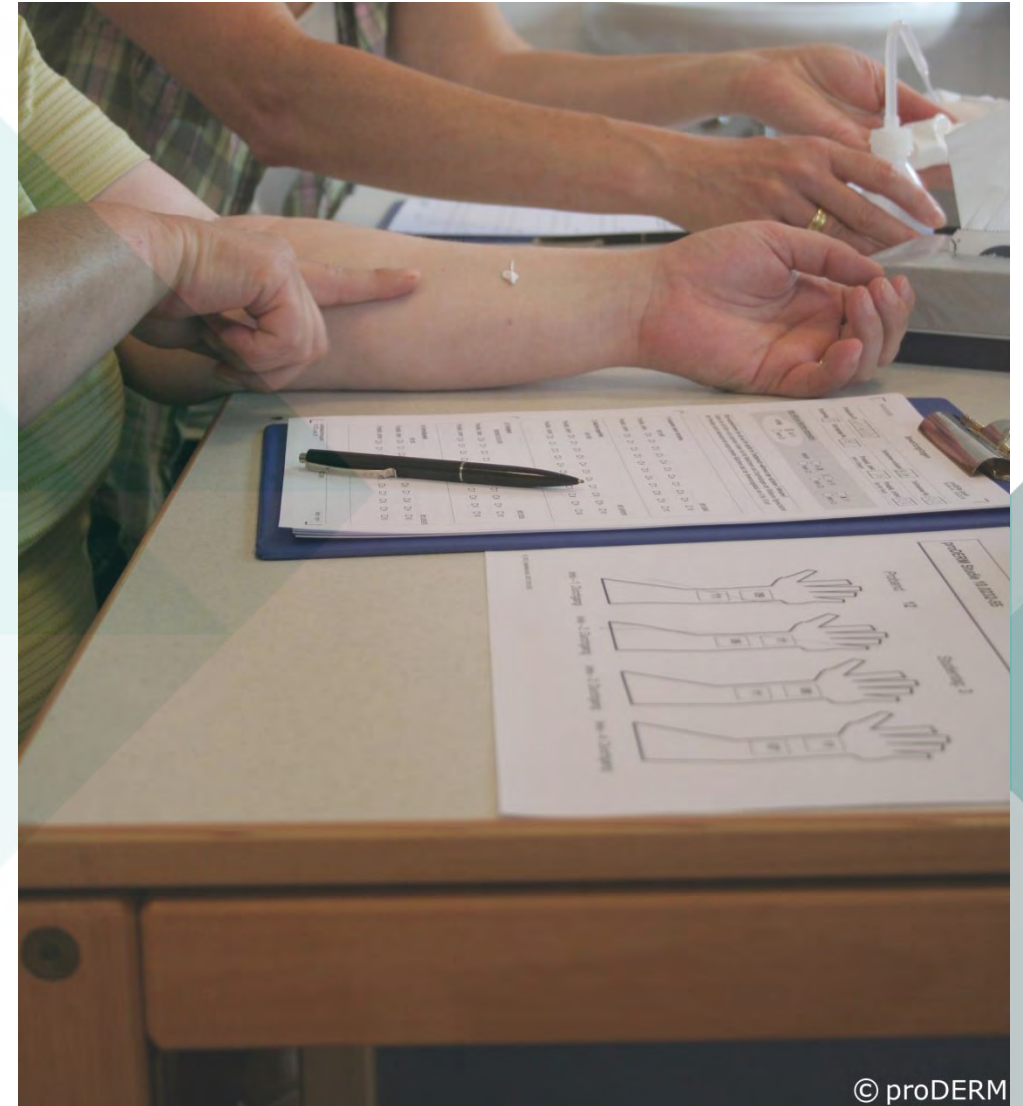
Presentation of images for assessment of defined parameters





# Self assessments by test subjects

- Realistic in-use testing on target population
- Assessment of parameters the consumers observe or feel
- Consumer perception
- Valuable data on product benefits
- Support findings of instrumental or biochemical studies





# Well-being and Quality of Life (QoL)



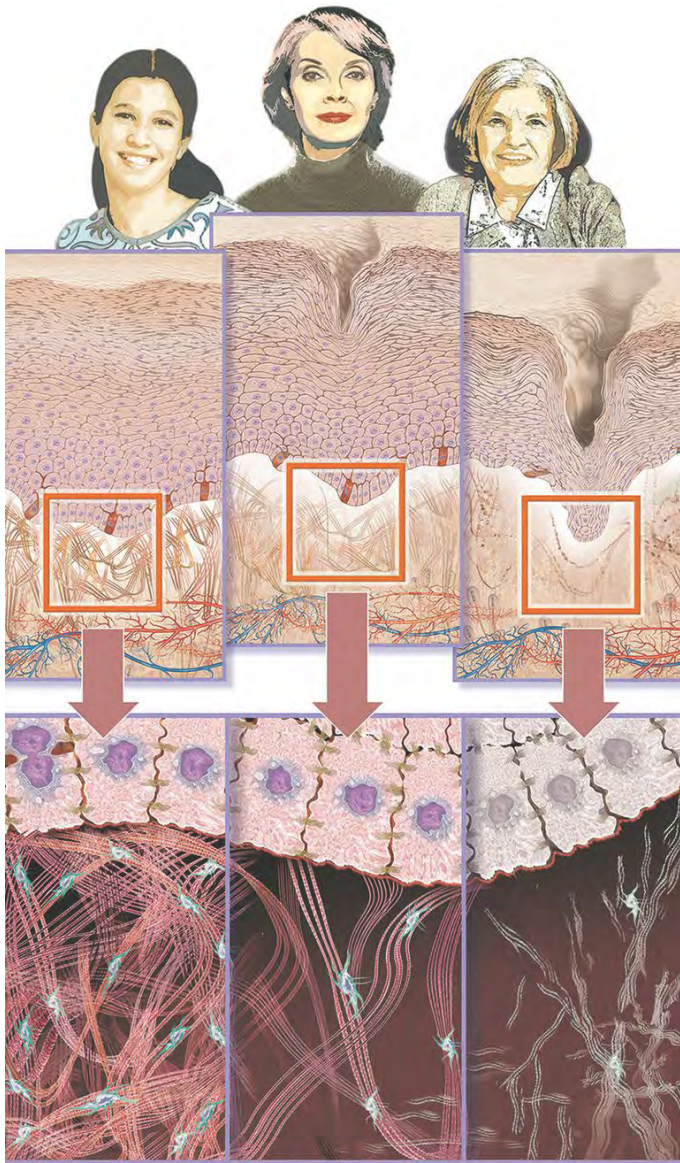
- Wellness, quality of life and well-being refer to the positive, subjective state
- In Dermatology the DLQI or the Skindex-16 are widely known
- There are also many disease-specific QoL questionnaires for patients with psoriasis, atopic dermatitis and acne.
- Quality of life questionnaires are often translated in many language but have sometimes to be validated due to cultural differences



# Assessments of Product Traits



- Personal perception of product efficacy and cosmetic properties on parameters that they can observe or feel
- Studies should involve realistic in-use testing in a relevant consumer base
- Wording should be clear
- Special attention should be paid to the wording of questions for which responses will be used to substantiate the claim
- Answers scale should be well balanced



# Aging on the epidermal level

## Epidermis

- Lipid content decreased (SC)
  - Flattening of dermal-epidermal junction
  - Number of enzymatically active melanocytes decreases by 8% to 20% per decade
  - Number of Langerhan's cells decreases
  - Capacity for re-epithelialization diminishes
- 
- Skin hydration ↓
  - Sebum secretion ↓
  - Elasticity ↓
  - Skin surface pH ↑
  - Protection and permeability barrier ↓
  - Pigmentation ↑
  - Decreased lipid content and altered lipid organization
  - Desquamation and proliferation ↓
  - Healing and immune function ↓



# Measurement of Dry Skin/Skin Hydration



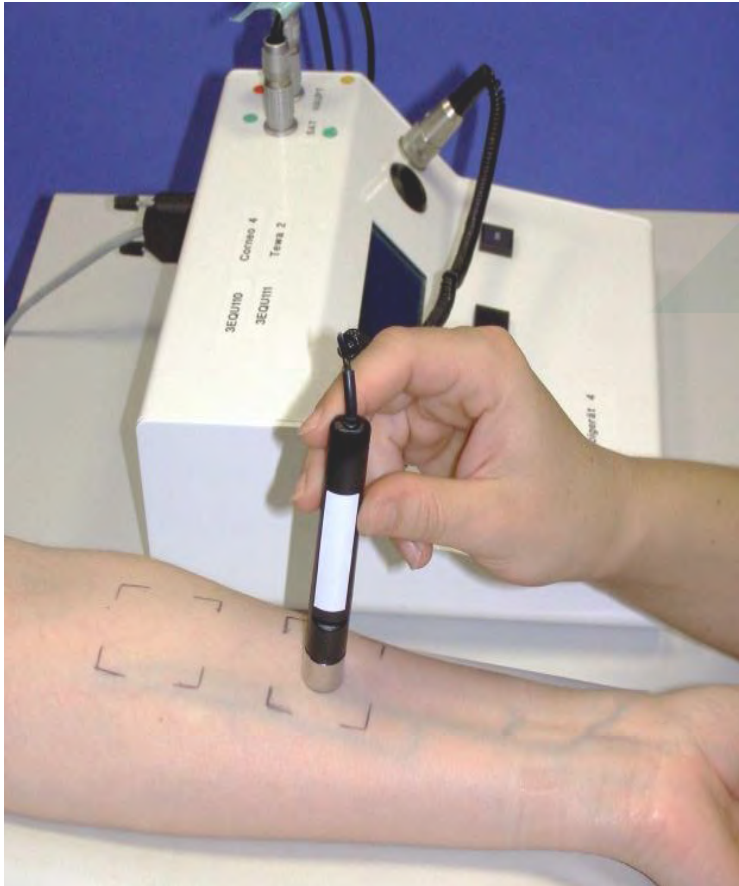
Selected Instrumental Measurements  
Corneometer, Epsilon, Moisture Mapping, Raman Spectroscopy,  
Dermalab Hydration

## Claims

- Moisturizing
- Hydrating
- long lasting protection
- prevent (protect) skin (dry skin)



# Corneometer



- Measurement of stratum corneum hydration
- Possible claims:
  - Instant hydrating effect
  - Long-term hydration
  - Hydrates the skin up to 24h
  - Moisturizing

- Corneometer CM 825 (Courage & Khazaka)



# Epsilon (Capacitance Measurement & Imaging)



Contact imaging of human skin, hair by capacitance measurement

- Equipped with 76800 sensors with a size of 0.4 x 0.4
- Test area of 12.8 x 15 mm
- Possible Claims
  - Sweat reducing
  - Moisturizing/ instant/long-term Hydration
  - Wrinkle reduction
  - Anti-Aging

- Epsilon E100



# Measurement of Skin Barrier Integrity



**Selected Instrumental Measurements**  
Tewameter, Aquaflux, Dermalab

**Selected Methods**  
Lipbarvis, Analysis from Swabs, Lipotype

## Claims

- Skin protection
- Repairs skin barrier
- Restores skin barrier function
- Preserves skin natural hydration
- Replenishes skin moisture barrier
- Lipid XYZ





# Tewameter

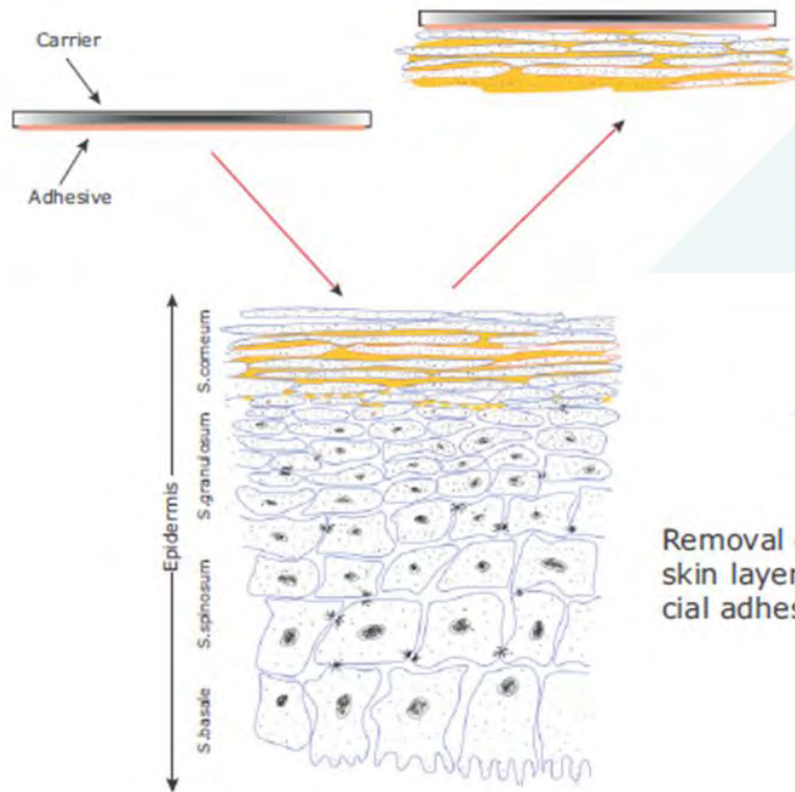


- Measurement of the integrity of stratum corneum (TEWL)
- Possible claims:
  - Skin protection
  - Repairs skin barrier
  - Restores skin barrier function
  - Preserves skin natural hydration
  - Replenishes skin moisture barrier

Tewameter-/ TM300

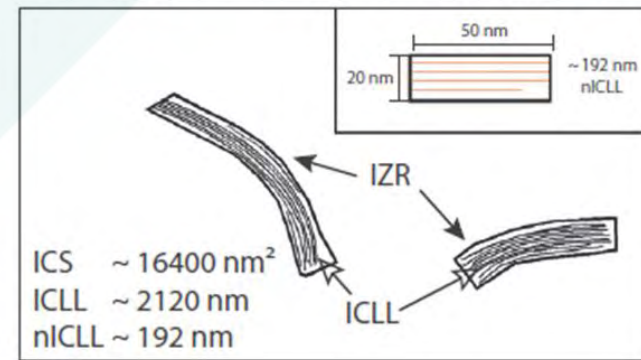
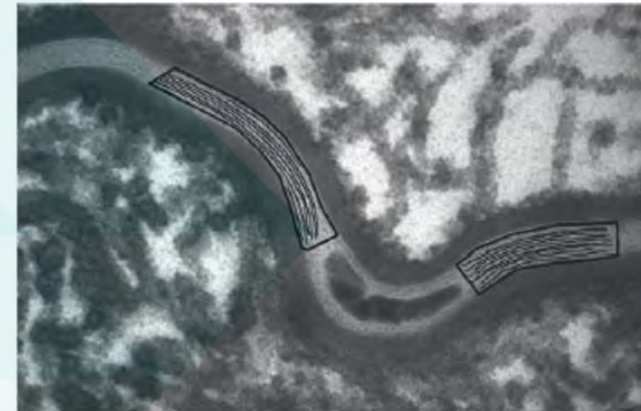


# Lipbarvis (Visualization of skin barrier)



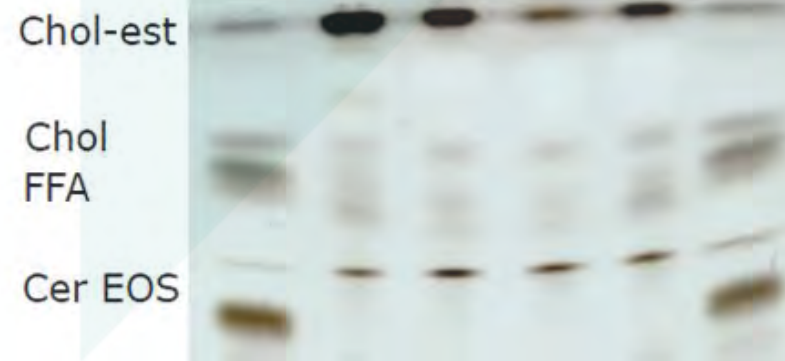
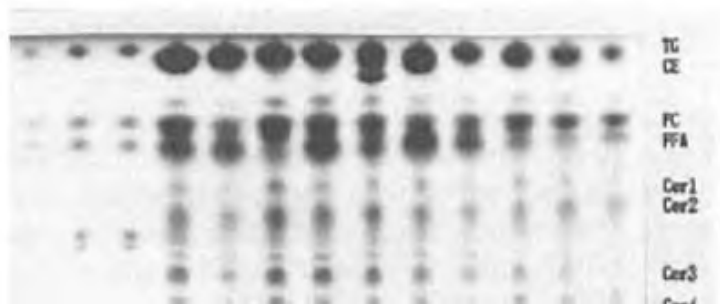
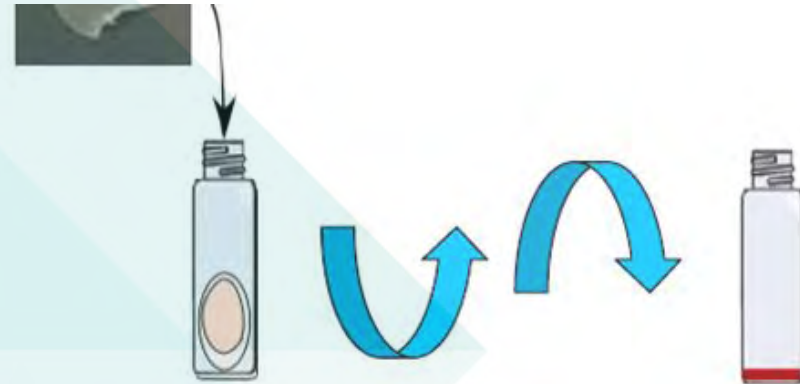
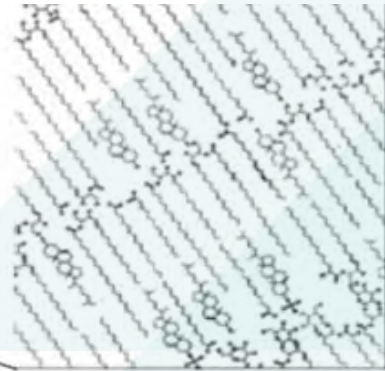
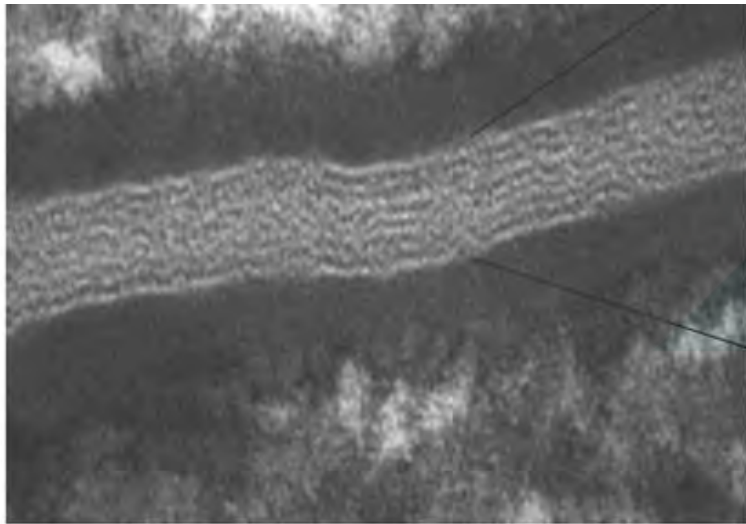
Removal of the uppermost skin layers through the special adhesive-carrier system

Morphometric analysis of epidermal barrier





# Lipbarvis LIP (Quantification of ceramides)





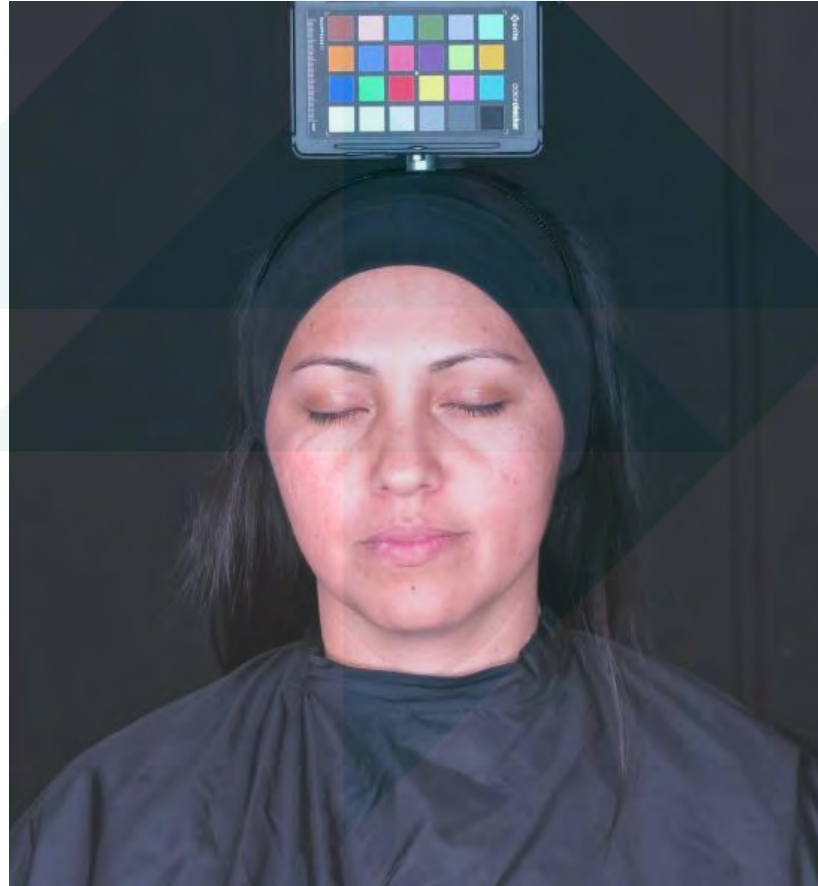
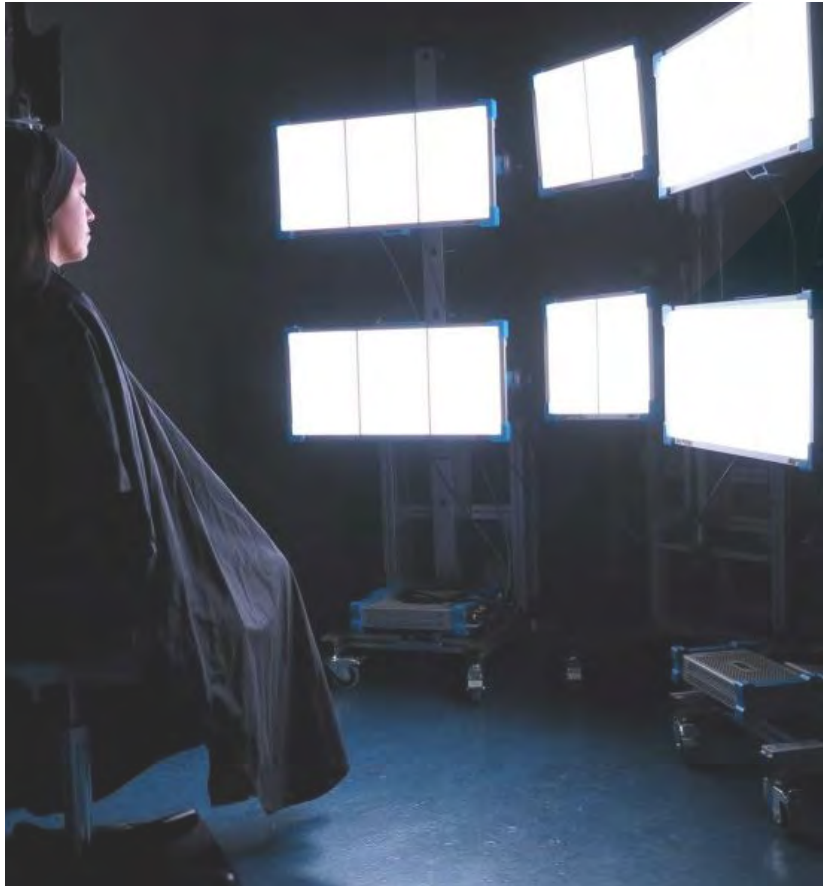
# Photo Documentation and Analysis



Selected Instrumental Measurements  
Visia CR, Visioface, Colorface, USRCliP, Dermlite,  
Visioscan, Leviacam, DermaScope Macis, HiRiS



## USR-CLiP (Unit For Standardized & Reproducible Clinical Photography)



- Multiple setups (e.g. full-face, feet,...)
- Hasselblad camera full-format 50 megapixel
- ARRI professional continuous light
- Overlay function
- Extraordinary accurate and reproducible colors
- Extraordinary sharpness and detail



# Standardized Facial Photography



## Colorface® Photobox

- 24 megapixel camera
- Daylight, polarized light (parallel or cross-polarized) and UV-light
- Front, 45° -left and right view
- Standardized color cards are photographed in images

## Visia CR2

- 21 megapixel camera
- Daylight, polarized light (parallel or cross-polarized) and UV-light
- Front, 37° -left and right view
- Standardized customized color cards are photographed in images



# Dark Circles



Automated segmentation of dark circle

- Images required:
  - Cross polarization images
  - Front face images
- Analysis parameters:
  - Morphological parameters : area
  - Color of dark circles:  $L^*$ ,  $a^*$ ,  $b^*$ ,  $ITA^\circ$ ,  $IWA^\circ$  Newton
  - Color of the surrounding skin:  $L^*$ ,  $a^*$ ,  $b^*$ ,  $ITA^\circ$ ,  $IWA^\circ$  Newton
  - Contrast between dark circles and surrounding skin:  $dL^*$ ,  $da^*$ ,  $db^*$ ,  $dITA^\circ$ ,  $dIWA^\circ$  Newton

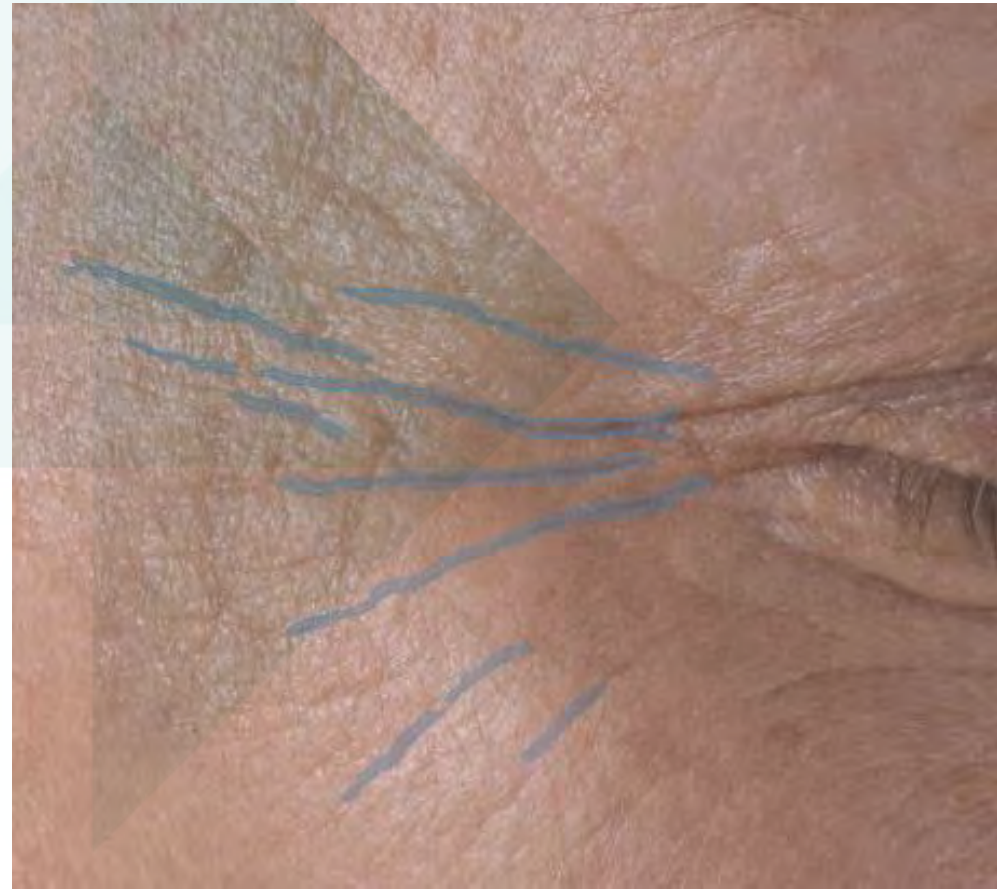
Automated  
segmentation  
of dark  
circle



# Wrinkles



Detail magnification



Automated segmentation of wrinkles





# Measurement of Skin Color



Selected Instrumental Measurements  
Photography followed by image analysis, Chromameter,  
Spectrophotometer

## Claims

- Skin Brightening
- Reduces the appearance of age spots
- Dark spot corrector
- More even skin complexion
- More homogeneous skin



# Pigmented Spots



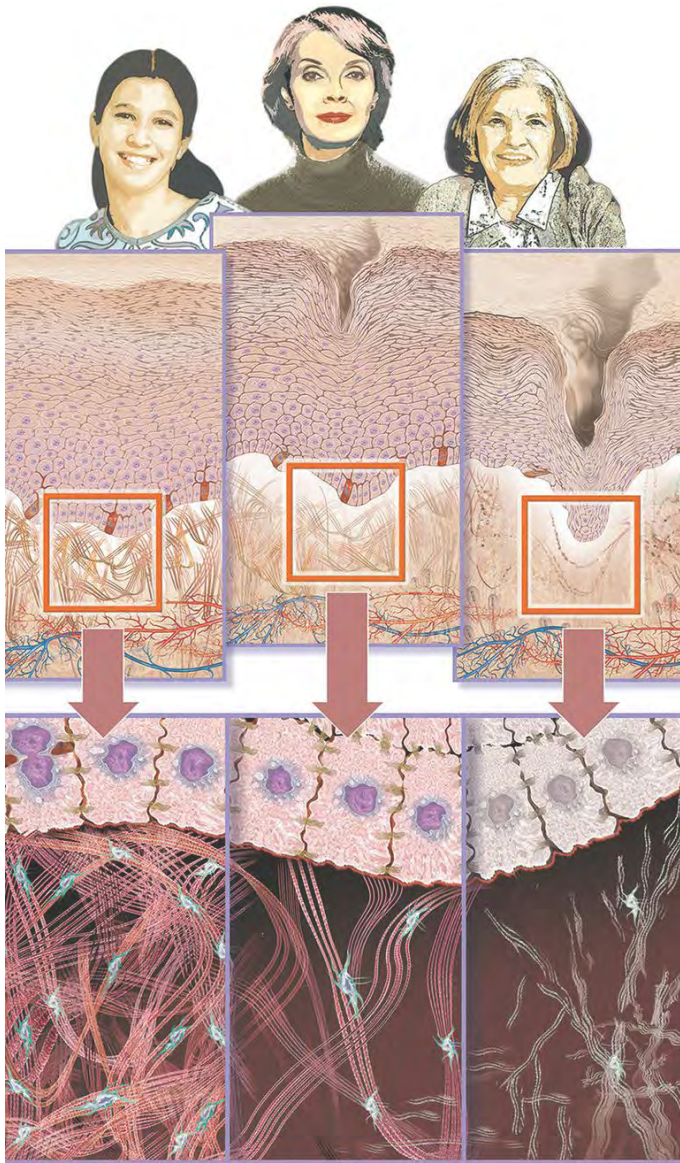
Colorface image (cross-polarized)



Single spot analysis



All pigment spots analysis



## Aging on the dermal level

### Dermis

- Decrease in thickness
- Decrease in thickness (atrophy)
- Vascularity decreases as does cellularity
- Decrease in collagen synthesis
- Pacinian and Meissner's corpuscles degenerate
- Structure of sweat glands becomes distorted, numbers of functional sweat glands decreases
- Elastic fibers degrade
- Decrease in number of blood vessels
  
- Degradation of Collagen and Elastin ↓
- MMP-1 ↑
- HA-production ↓
- Glycation ↑
- Deep Wrinkles ↑
- Sagging ↑



# Measurement of skin roughness and elasticity



## Selected Instrumental Measurements

3D: DermaTOP, Primos, AEVA-HE, Replica

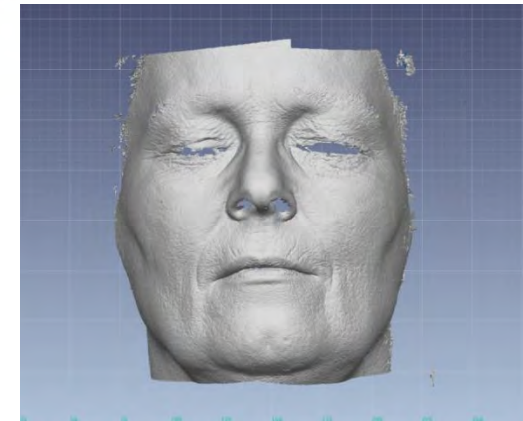
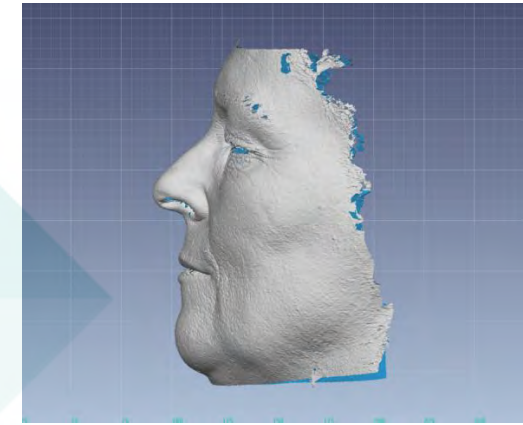
Elasticity: Cutometer, Cutiscan, DynaSkin, Dermal Torque Meter

## Claims

- Reduces fine lines and wrinkles
- Smooths wrinkles
- Anti-wrinkle cream
- Slows appearance/look of aging
- Skin Firmness



# Measurement of Facial Wrinkles by Fringe Projection (AEVA-HE)





# Touch Free Measurement Skin Firmness: DynaSkin

before, during and after

Name

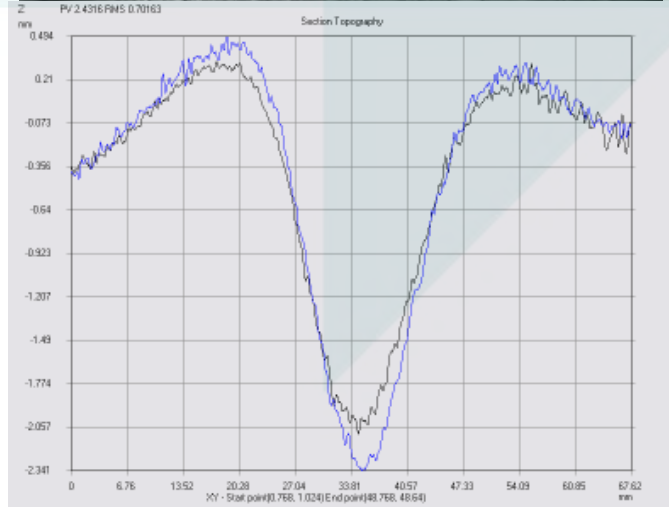
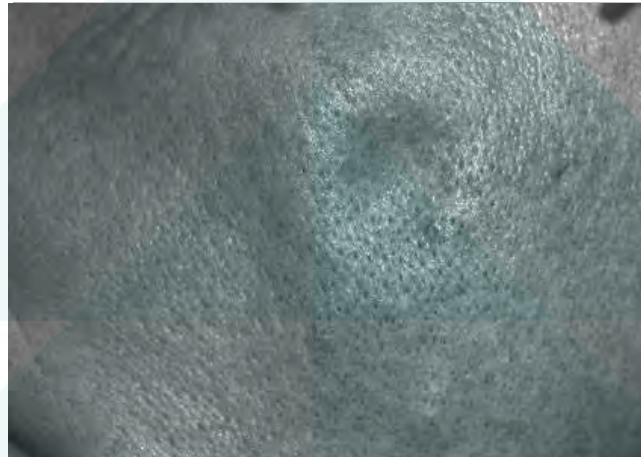
Volume m

Circumfe  
mm

Area mm

Max dept  
mm

Mean dep  
mm





# Cutometer



- Measurement of skin elasticity, firmness
- Possible claims:
  - Improves Firmness
  - Improves Elasticity
  - Tonicity and/or Suppleness
  - Skin feels/appears lifted

- Cutometer (Courage & Khazaka)



# Assessment of intrinsic aging and photoaging via dermal water content and collagen network



## Selected Instrumental Measurements

Raman Spectroscopy, Vivascope, LC-OCT, Vivosight, Ultrasound, Multiphoton, Suction blister roof and fluid

## Claims

- Fill-up Therapy
- Collagen Nutrition





# Confocal Raman Microspectroscopy

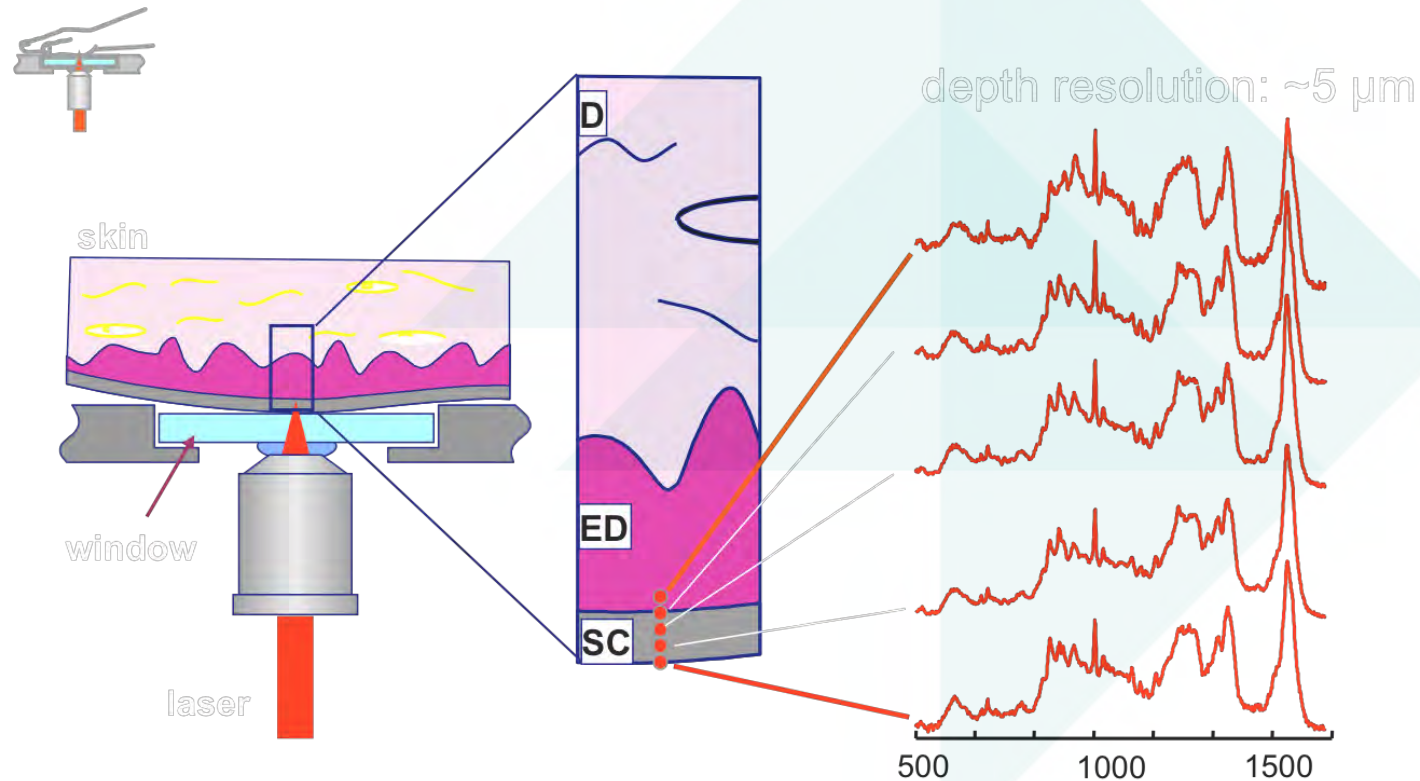


Raman spectroscopy enables **non-invasive in vivo** analysis of the molecular composition of the skin

- Water concentration profiles from skin surface to dermis in minutes
- Determination of Natural Moisturizing Factors concentration or other molecules in the stratum corneum and below
- Studies of the penetration of topically applied materials



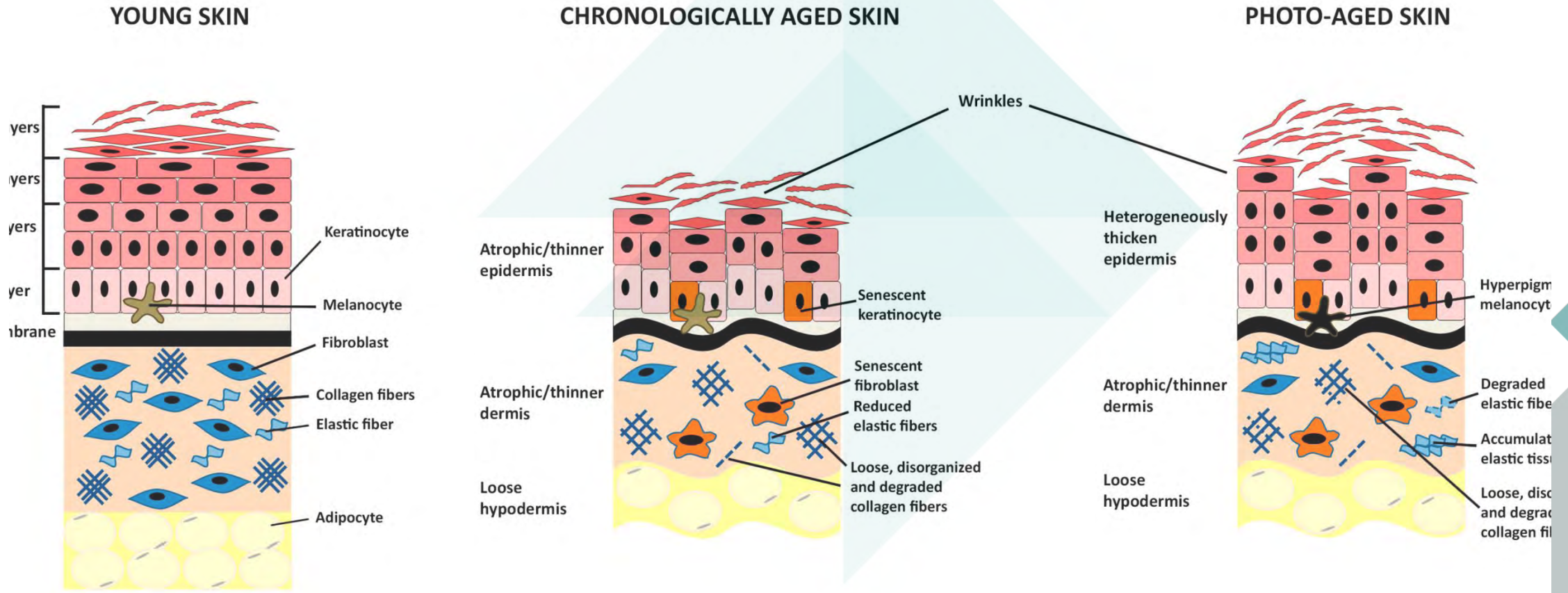
# Confocal Raman MicroSpectroscopy (CRMS)



Up to 200  $\mu\text{m}$  in depth  
Best depth resolution:  $\sim 3 \mu\text{m}$

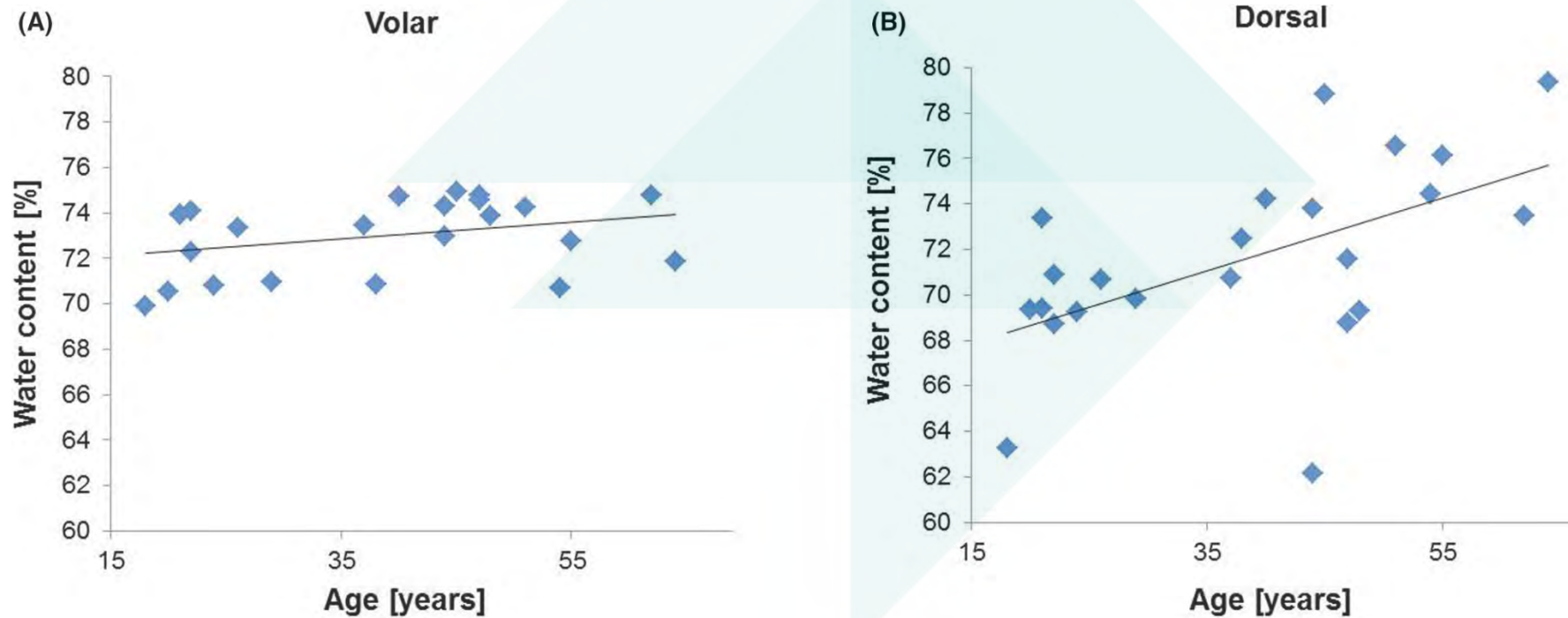


# Confocal Raman MicroSpectroscopy - Collagen



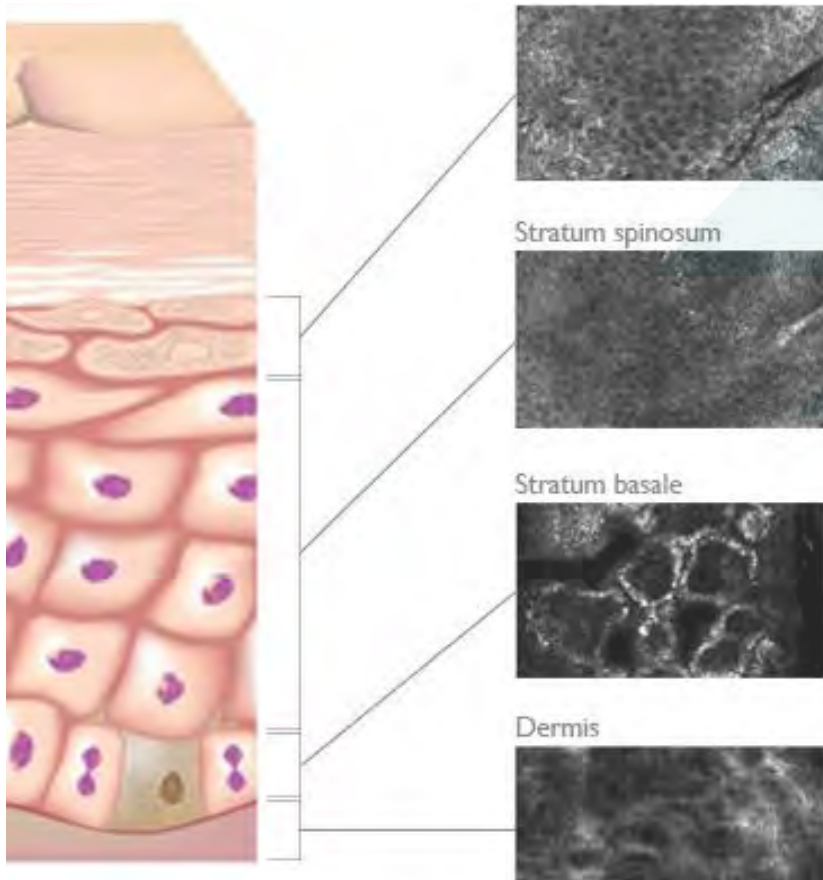


# Correlation of water content of volar dermis at a depth of 130 to 150 $\mu\text{m}$ vs age for subjects





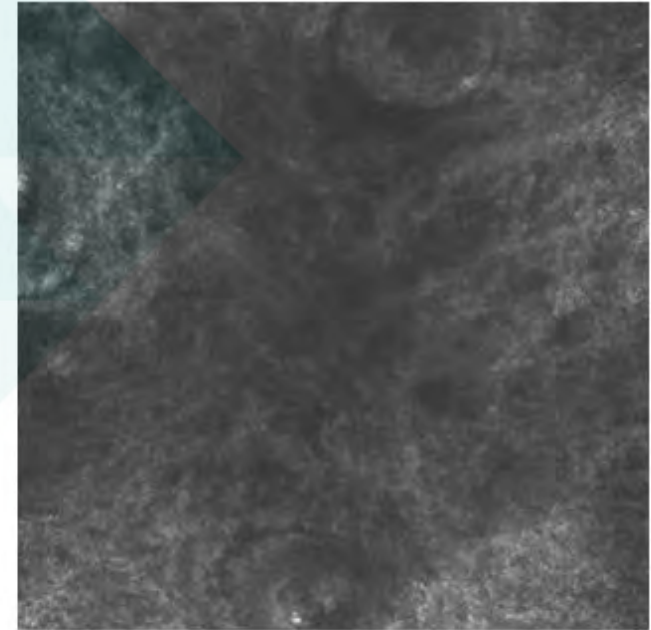
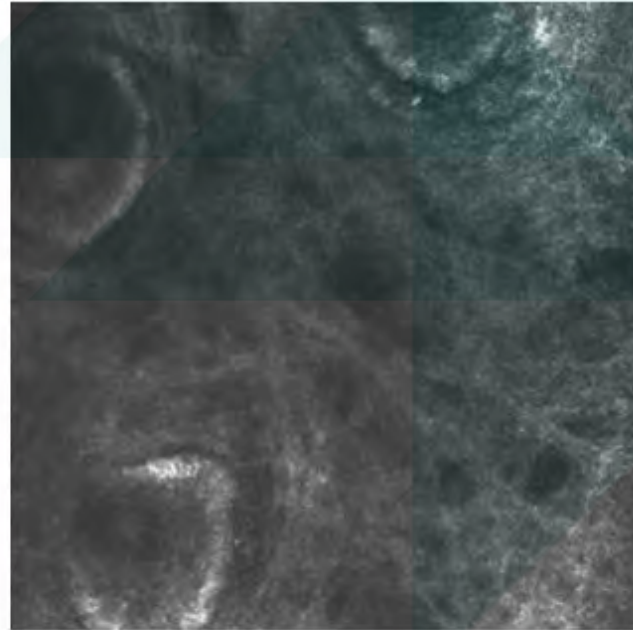
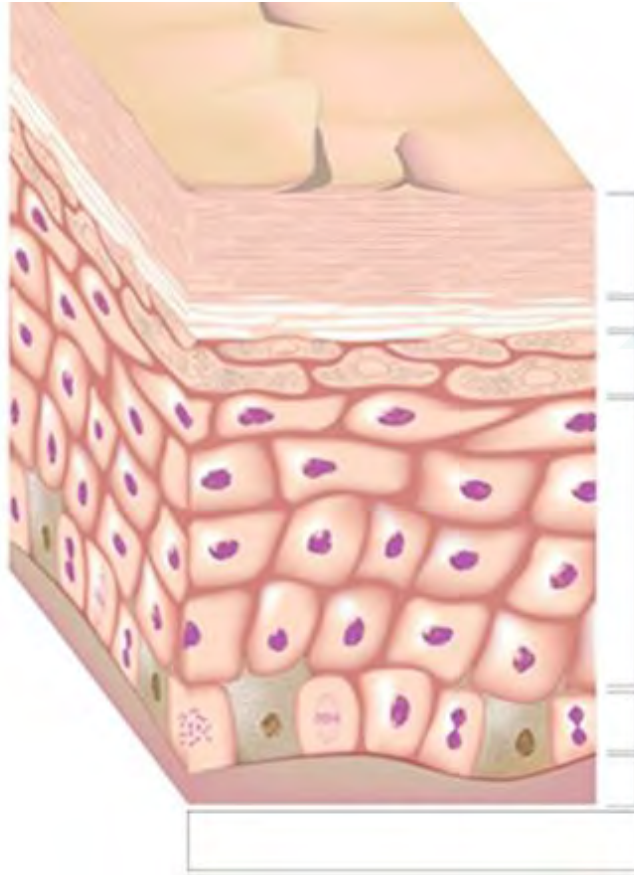
# Confocal Microscopy (Vivascope)



- A laser scanning confocal microscope that produces cellular images of exposed epithelial tissue
- Imaging area between 500x500  $\mu\text{m}$
- **Non-invasive assessment:**
  - Aging, dermal papillae structure
  - Stratum Corneum Thickness
  - Epidermal Thickness
  - Pigmentation, Melanin Granula
  - Collagen Structure – Fibre Network



## Structure Comparison (Collagen)

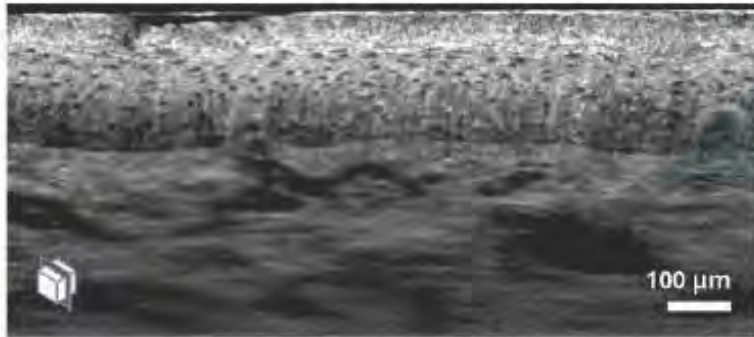


Product effect from day 1 to day 85 in the structure of the reticular dermis

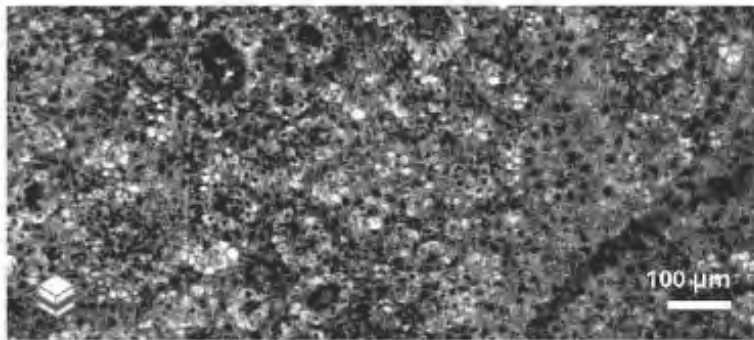


# LC-OCT

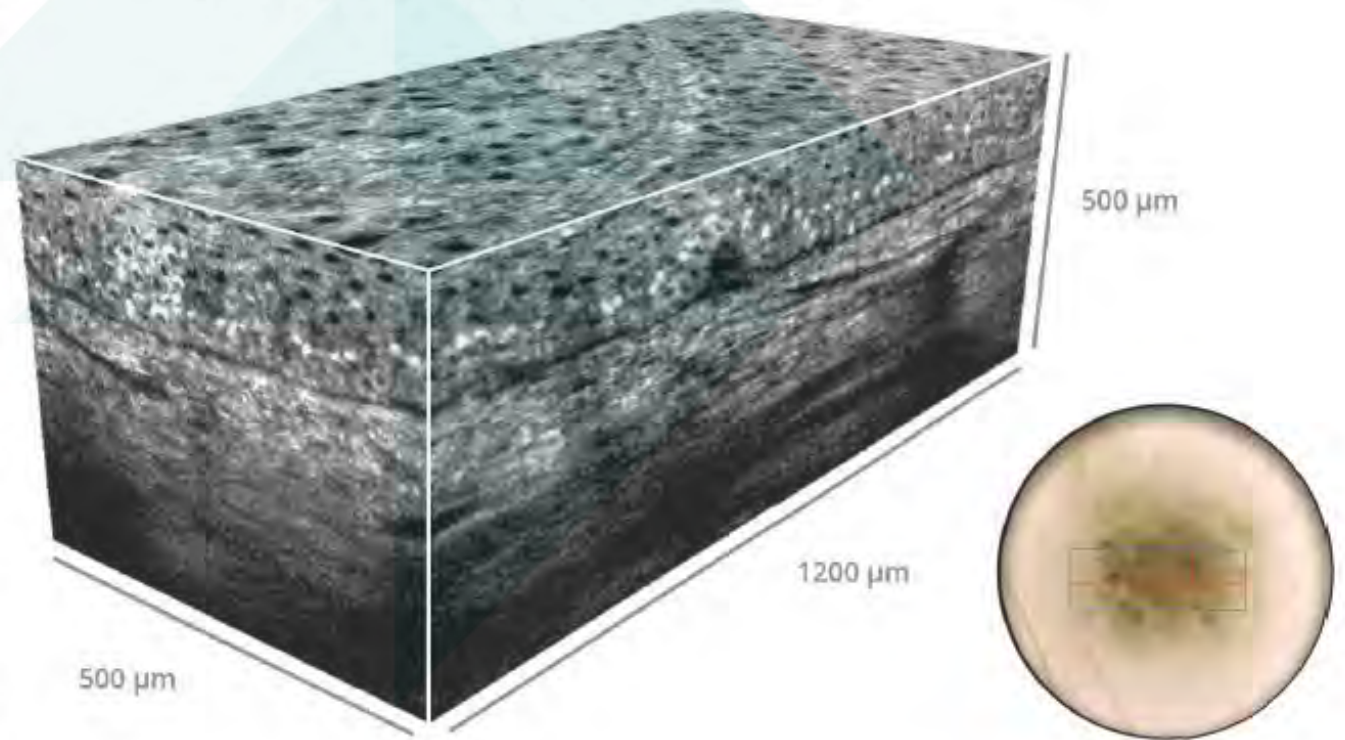
Vertical live mode



Horizontal live mode



3D stack with isotropic cellular resolution



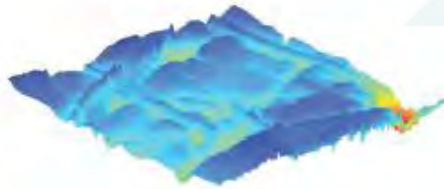


# What can be measured?

## SKIN LAYERS & DEJ

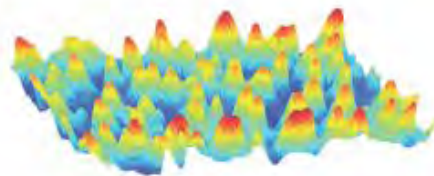
### SKIN LAYERS

*Thickness of  
Stratum Corneum,  
Stratum Spinosum,  
Superficial Dermis*



### DERMO-EPIDERMAL JUNCTION (DEJ)

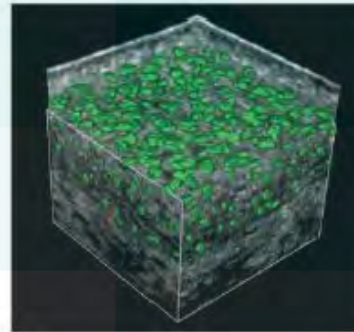
*DEJ & papillae  
morphology,  
size and distribution*



## CELLULAR

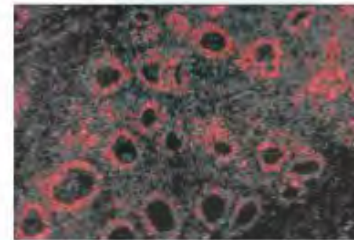
### KERATINOCYTES

*3D quantification  
of keratinocyte nuclei  
size and density*



### MELANIN

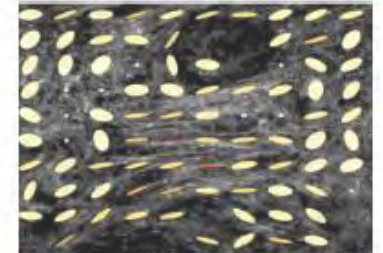
*3D quantification  
of melanin density  
and distribution*



## TISSULAR

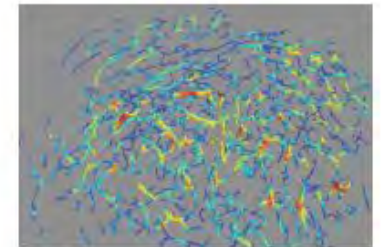
### COLLAGEN

*Collagen density  
Fiber orientation*



### BLOOD VESSELS

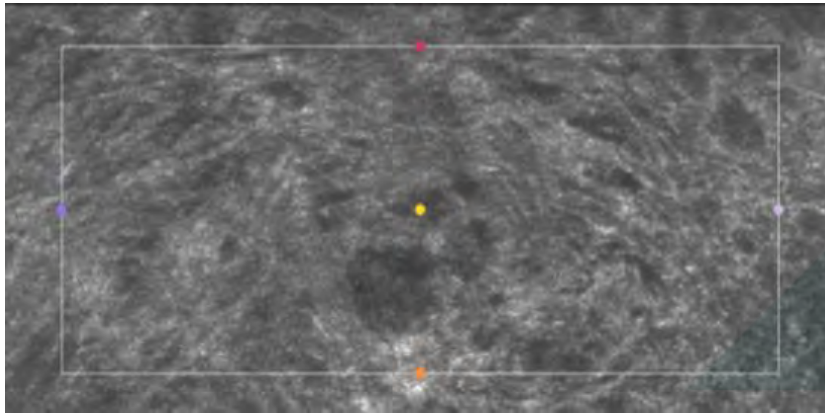
*Size of the vessels  
Vascularization  
organization*



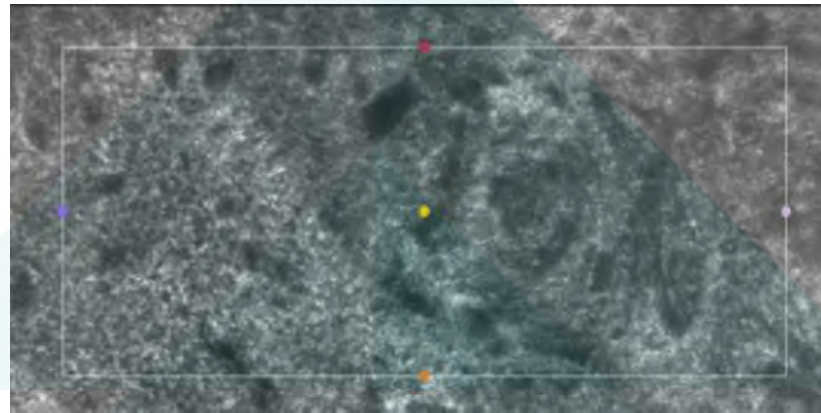




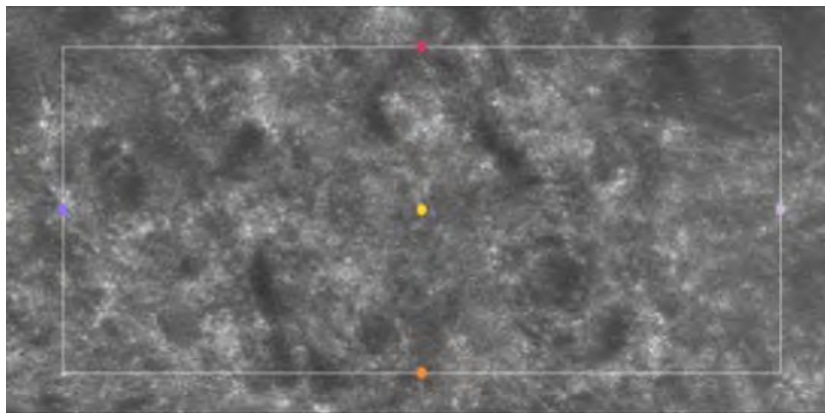
# Collagen structure of young and old subjects



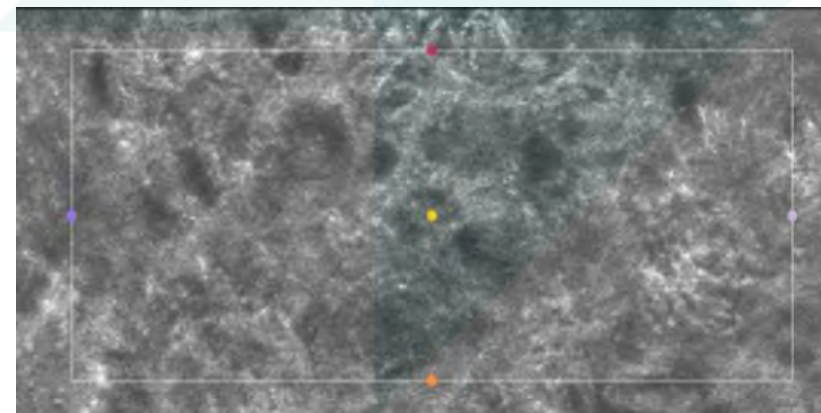
Subject; Age 62, dorsal photoaged



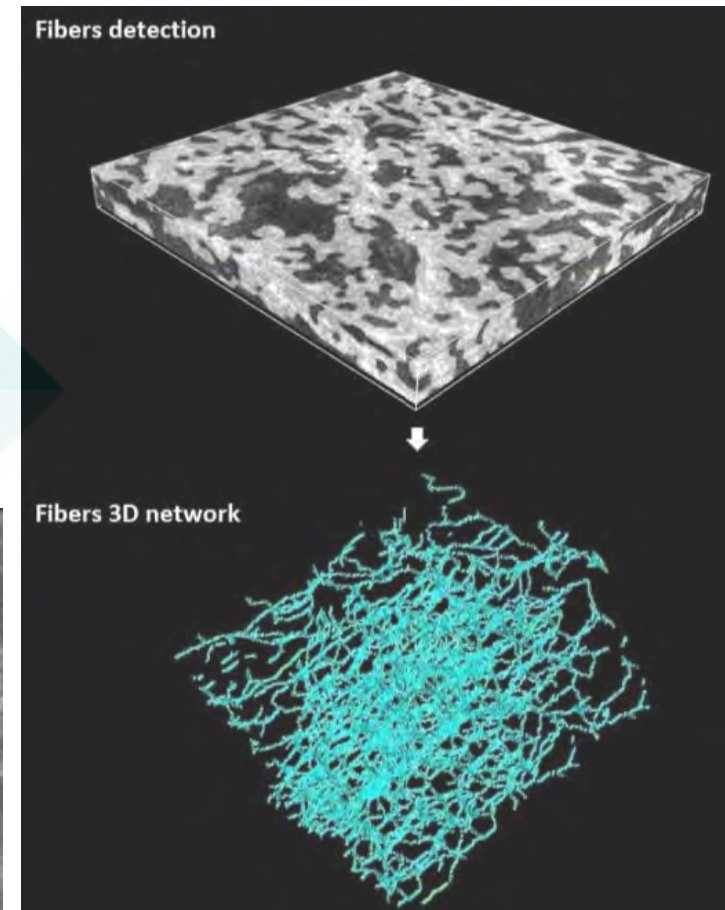
Subject; Age 62, volar



Subject; Age 7, dorsal



Subject; Age 7, volar

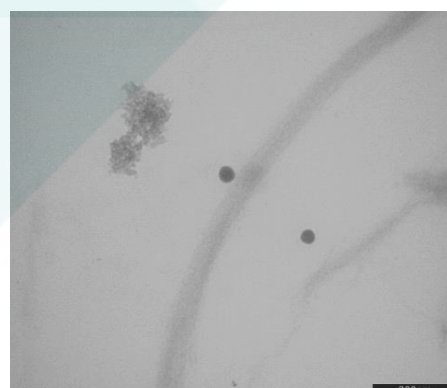
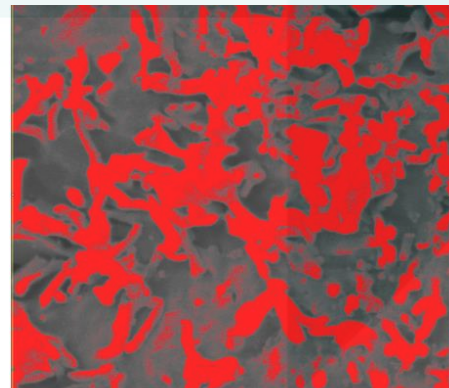
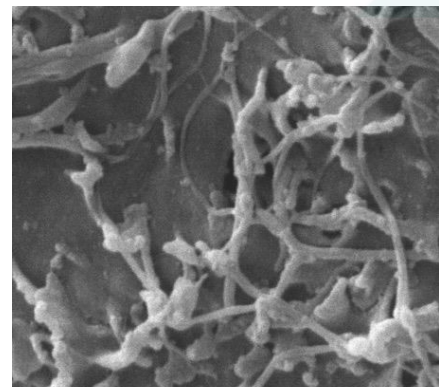
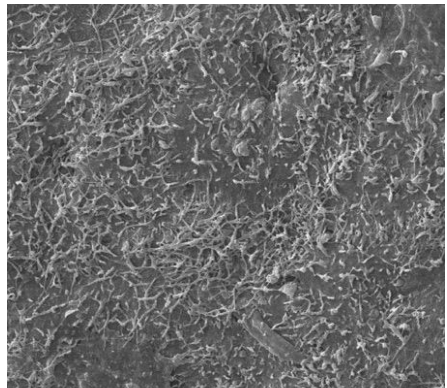
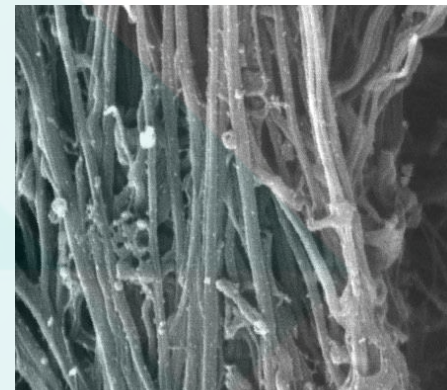
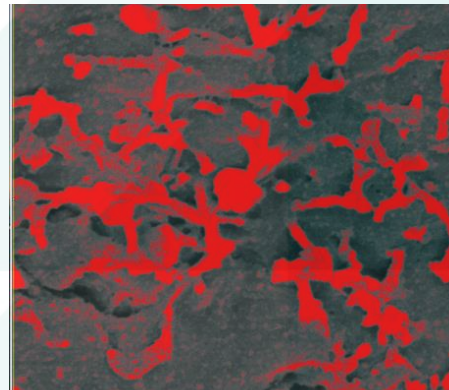
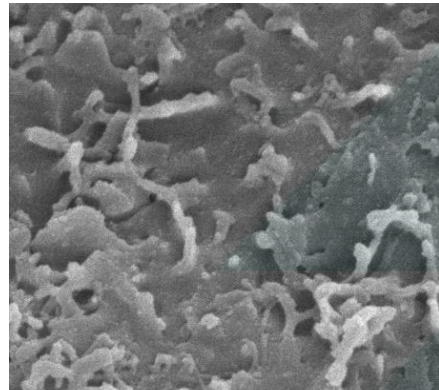
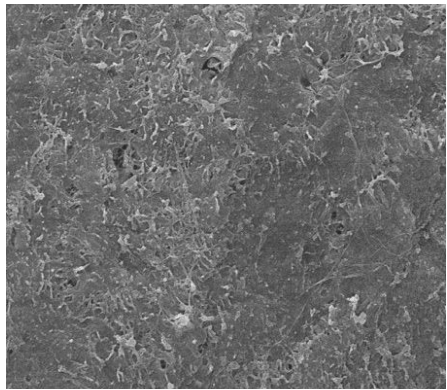


Collagen fibers 3D network



# TEM evaluation of suction blister roofs

## Analytics from suction blister fluid



Untreated/Treated

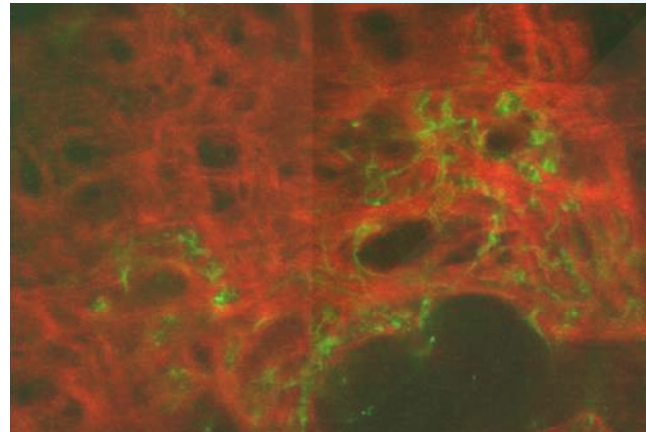
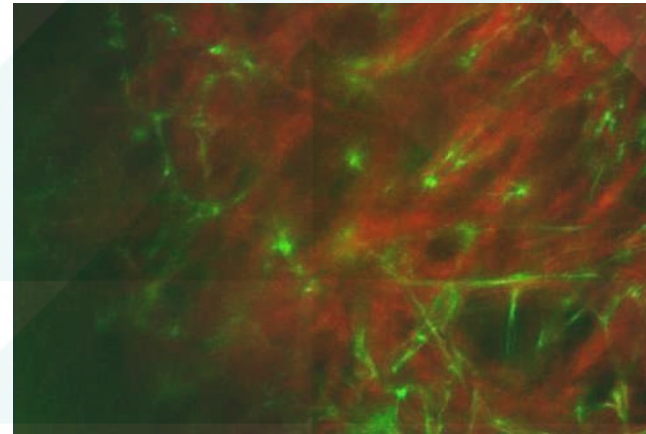
Untreated/Treated

Untreated/Treated  
quantified

Immunostaining with gold-  
antibodies



# Multiphoton Tomograph

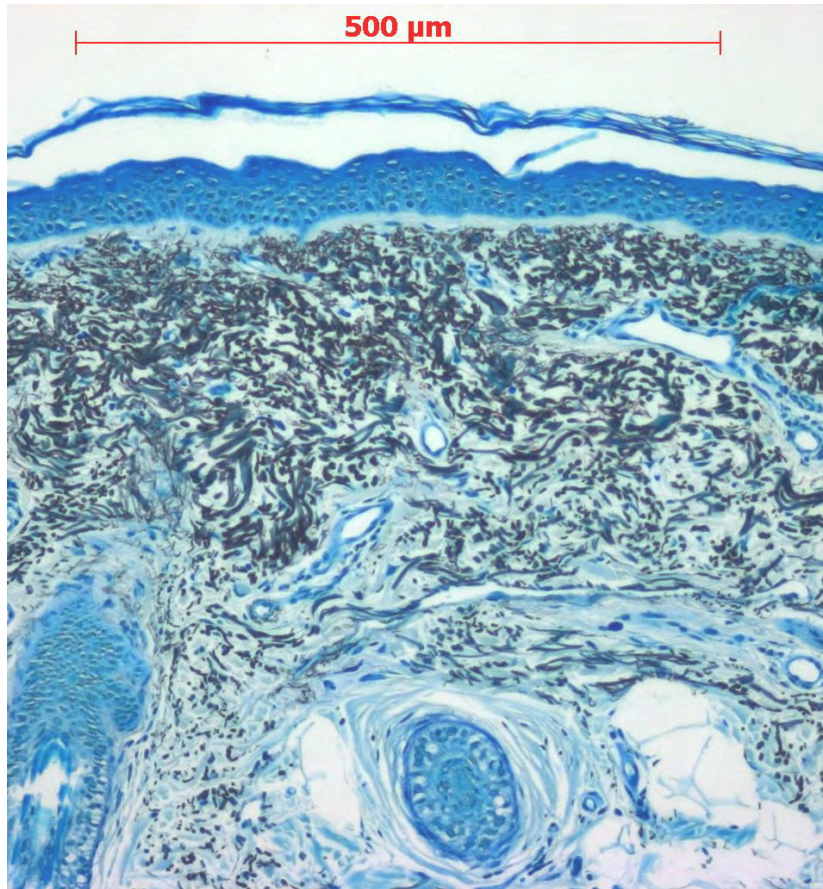


- The false-colored overlay of two signals demonstrates
- morphological structures of dermal layers:
- autofluorescence of elastin and collagen crosslinks (green)
- and collagen SHG-signal (red).
- Optical biopsies

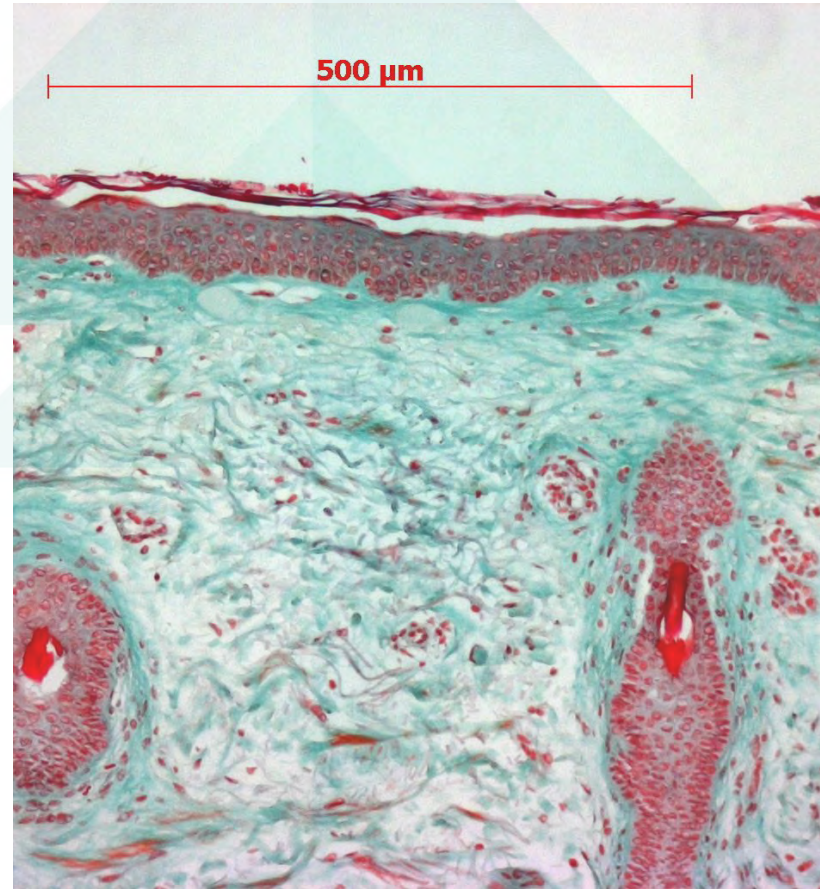
• Photo: Paul



# Biopsy stainings for Collagen and Elastin



Orcein staining

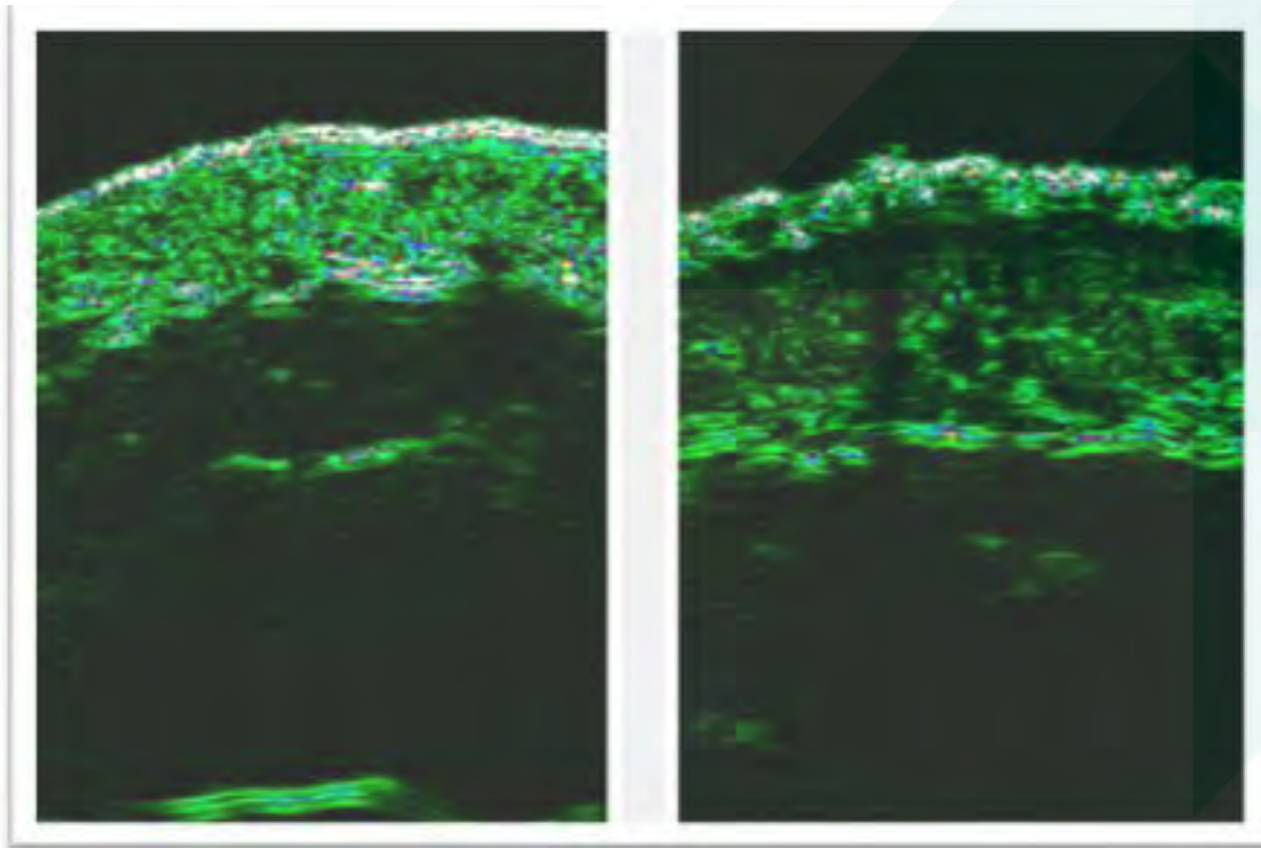


Masson-Goldner staining

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



## 22 MHz-Ultrasound Measurement on Photo Damaged Skin: „Echo-poor Region“ due to Degraded Fibres in the Dermis



Normal skin (outer forearm)

Photoaging: Echo poor region  
below epidermal echo



# AGEs - Advanced Glycation End products



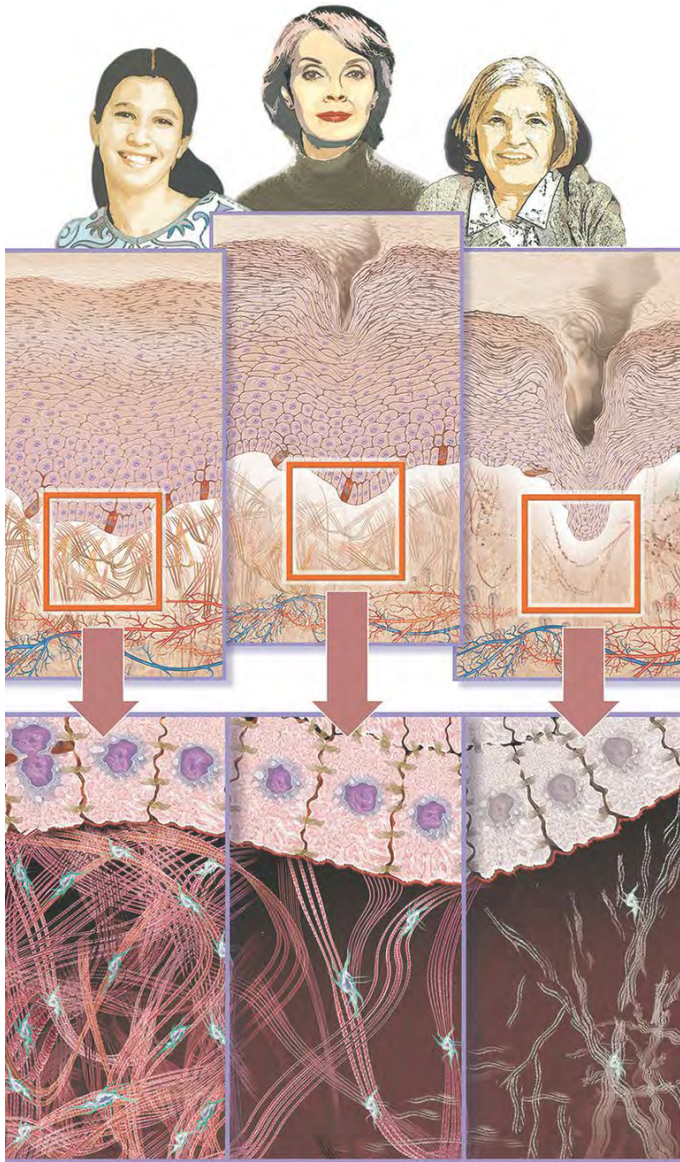
- The AGE Reader is a non-invasive monitoring device that uses ultra-violet light to excite autofluorescence in human skin tissue. The autofluorescence is from the level of Advanced Glycation End products (AGEs).
- As AGEs lead to the wrinkling of skin, they are an important target for the skin care industry. Most of the large international cosmetic companies have already developed creams and capsules targeting AGEs in the skin.

AGE Reader



# Aging on the subdermal level Hypodermis

- Change in distribution of subcutaneous fat.





# Measurement of Cellulite



Selected Instrumental Measurements  
Images, DermaTOP, FLPI, Thermography, Cutometer

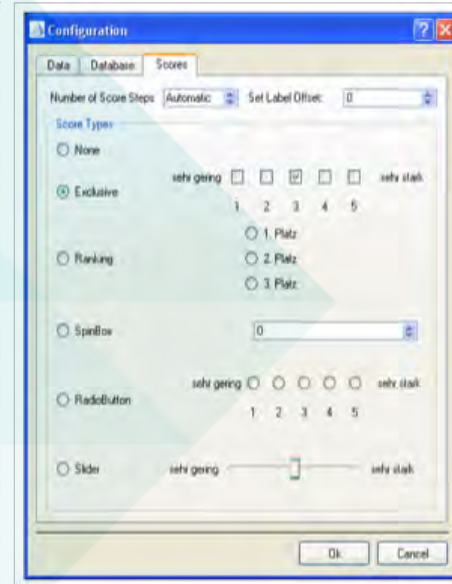
## Claims

- Firming
- Reduces the look of cellulite



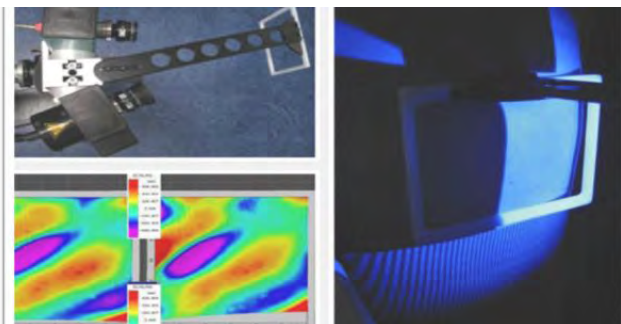
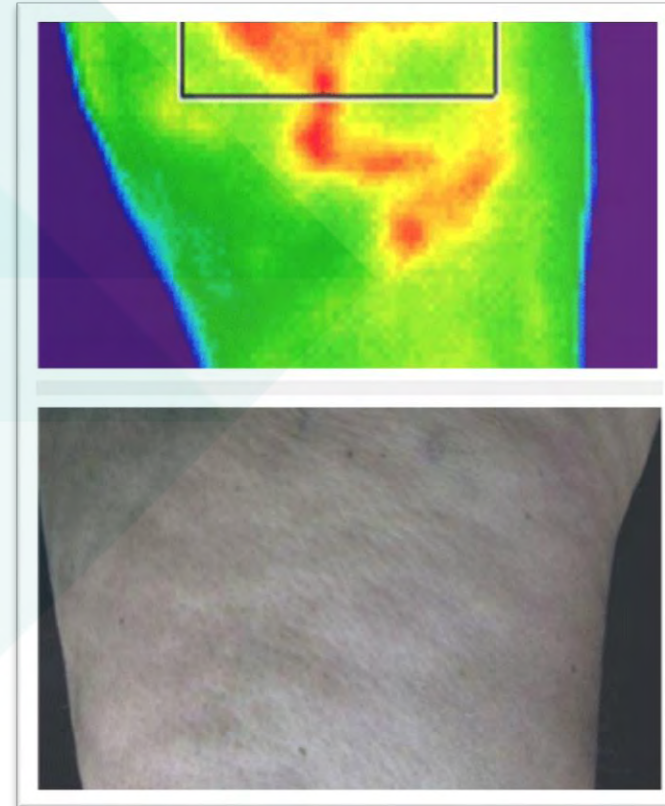
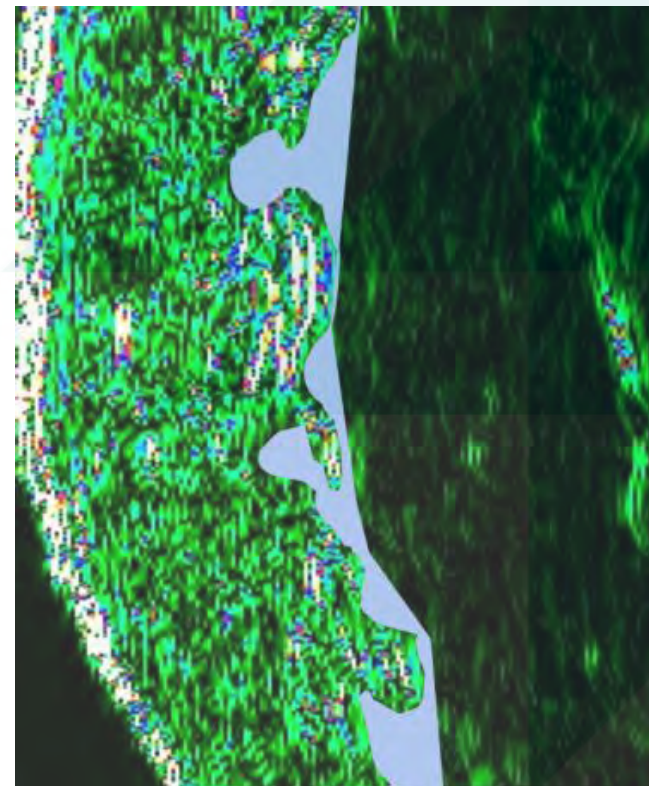
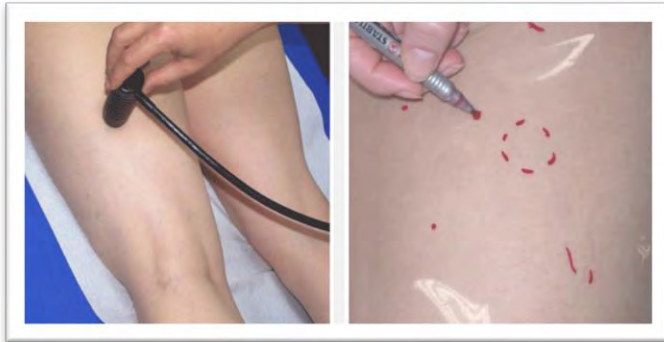


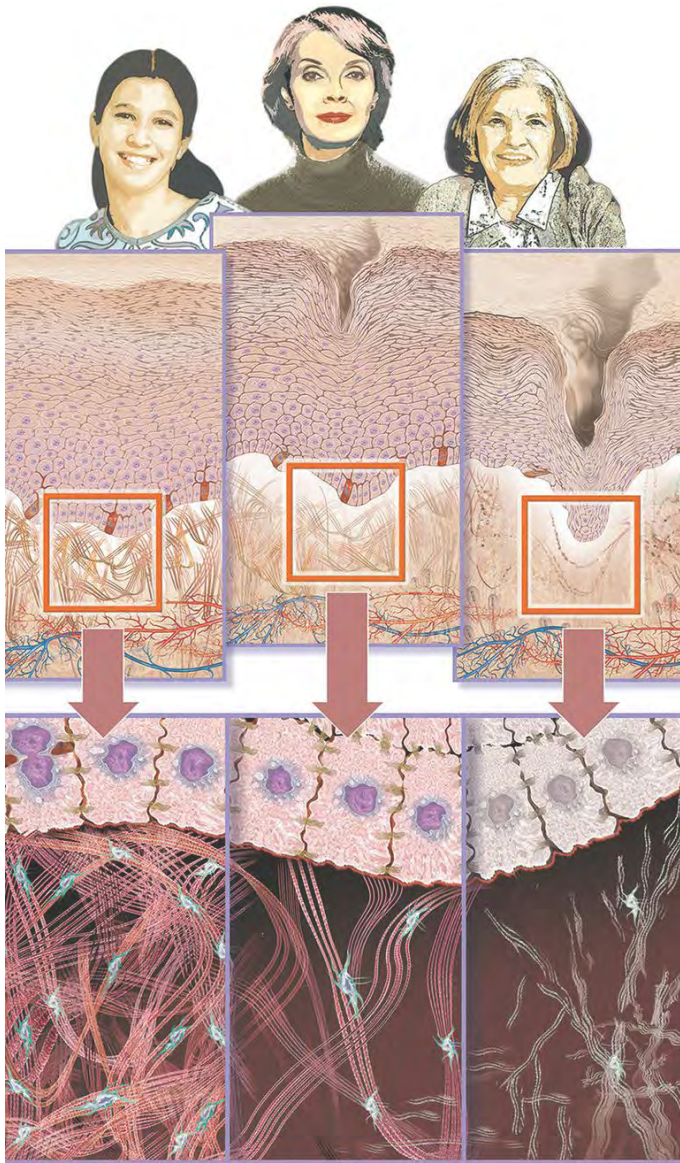
# Cellulite Photography Stand





# Further Methods





## Aging - Appendages

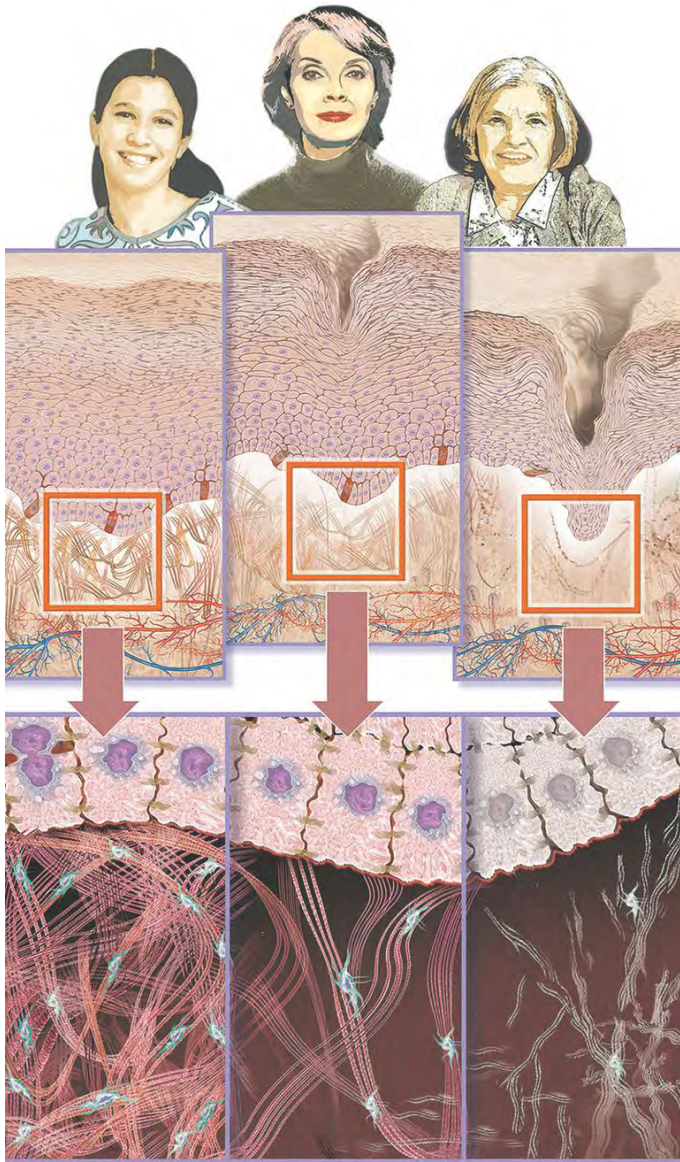
### Sebaceous, apocrine, and eccrine glands and hair follicles

- Hair loses normal melanin
  - Hair thinning
  - Decreased number of sweat glands
  - The nail plate becomes abnormal
  - Reduced sebum production
- 
- Sebum secretion ↓
  - Inflammaging ↑
  - Pore size ↑
  - Hair ↓



# Environmental stressors in skin aging

## Sun radiation





# Sun Protection Factors (SPF and WR) Testing and UV-A Protection (UVAPF)



## Static SPF

- ISO 24444
- Australian/New Zealand Standard Test Method AS/NZS 2604
- FDA Test Method

## UV-A Protection (UVAPF)

- 24442 (UVA in vivo)
- ISO 24443 (UVA in vitro)
- JCIA-Guideline
- Korean Method
- FDA

## WR

- ISO 16217:2020 and ISO 18861:2020
- Australian/New Zealand Standard Test Method AS/NZS 2604
- FDA Test Method



# IR-A Protection

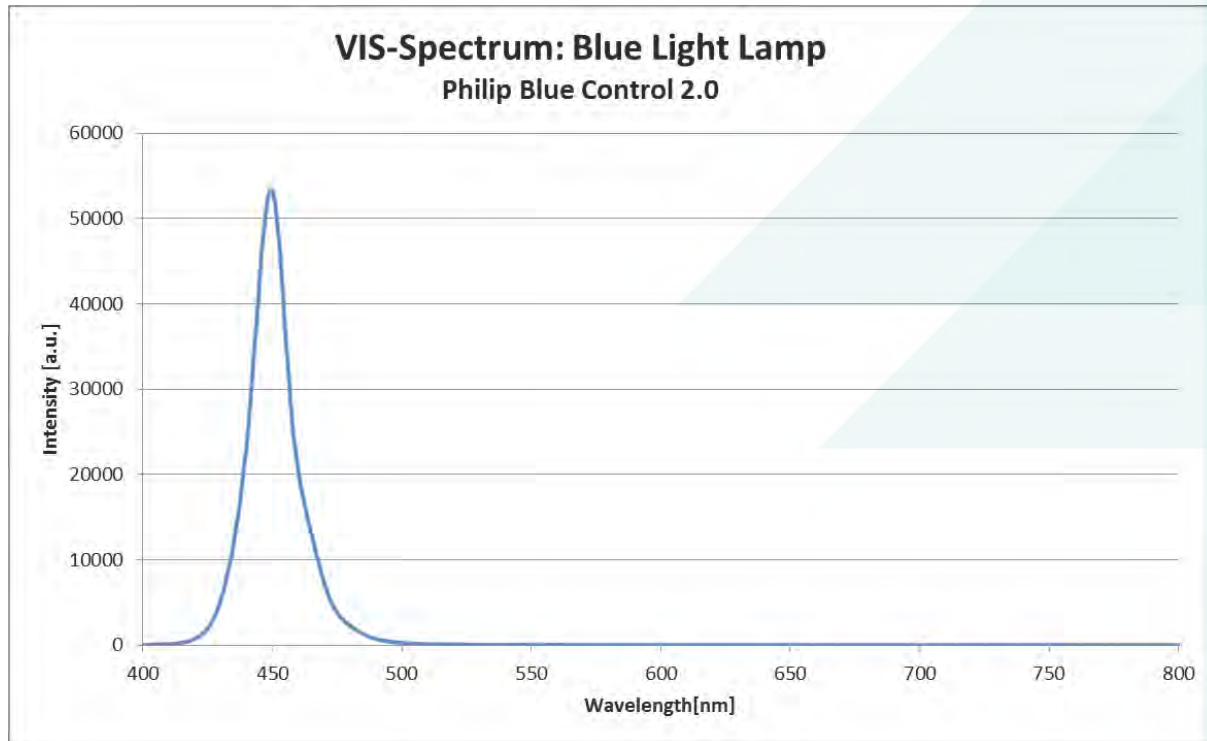


Biozoom

- FLPI and Thermography (Heat-Protection)
- MMP1/TIMP1 as a marker from suction blister fluid (ELISA)
- In vivo Carotenoid measurement by Multiple Spatially Resolved Reflection Spectroscopy (MSRRS – Biozoom)

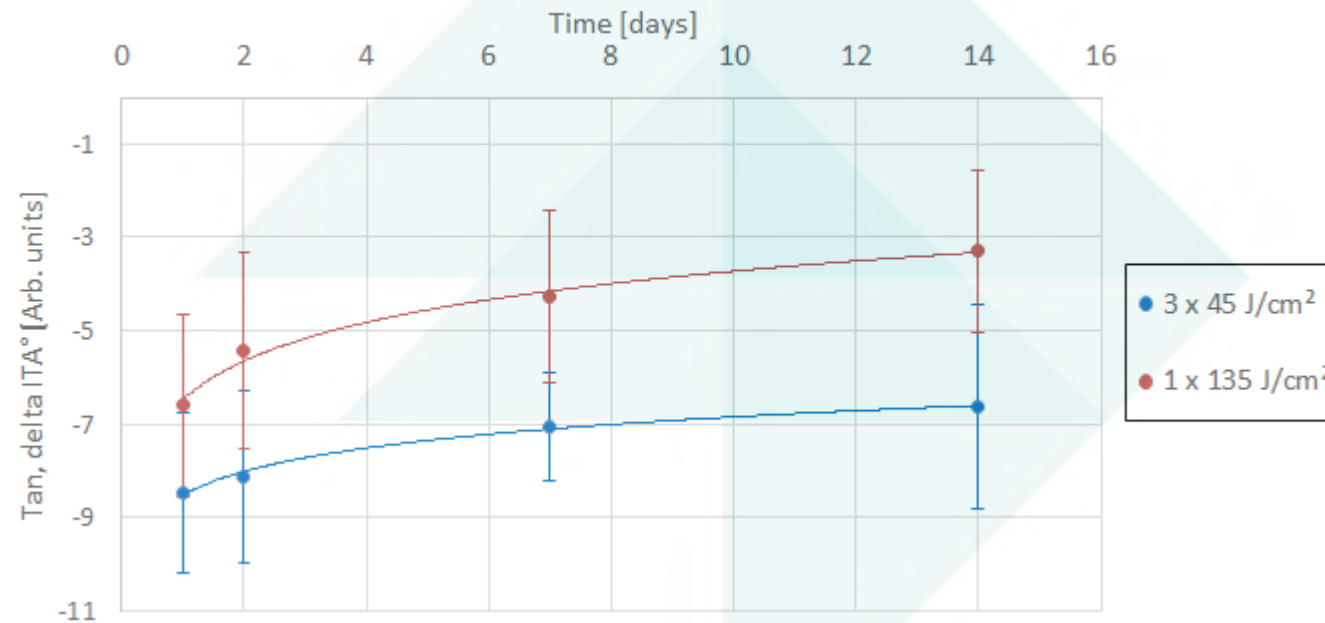


# Irradiation with Blue Light at proderm





# proderm study: Tan Formation and Development



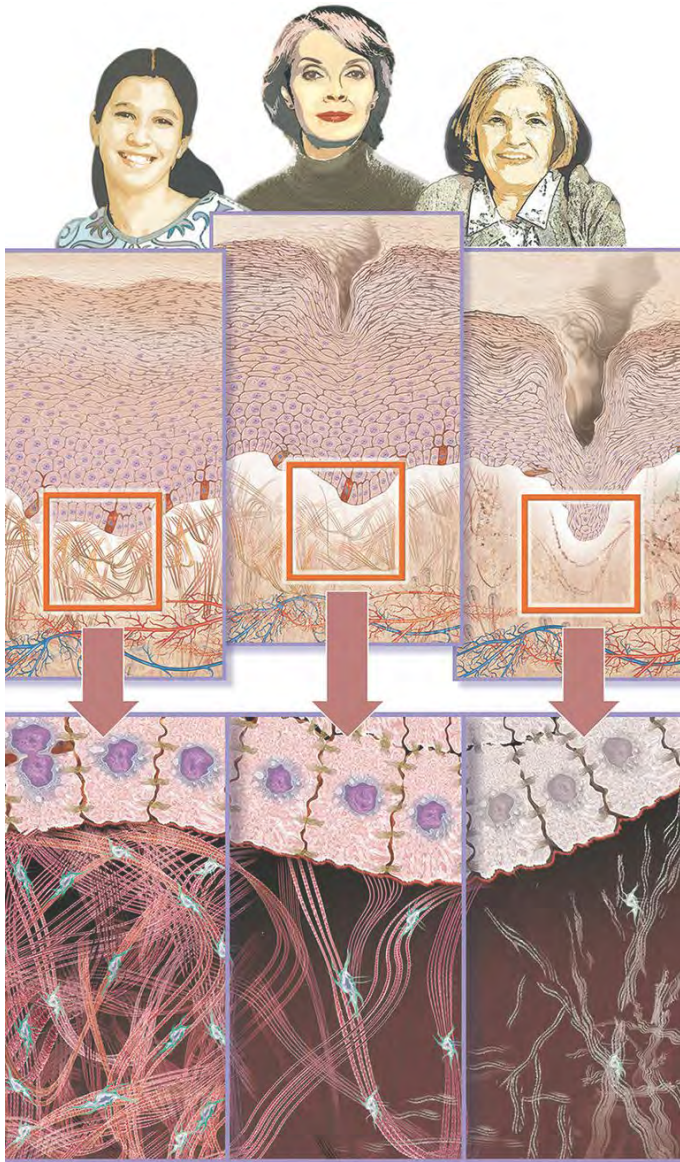
Three repeated doses led to a stronger tan response than a comparable single dose; Visible tan at about  $\Delta ITA^\circ = 2$





# Environmental stressors in skin aging

## Pollution/Tobacco





# The major components of air pollutions

## Toxic gases

- Sulfur dioxide
- Nitrogen oxides
- Photochemically derived ozone
- Volatile organic compounds

## Particulate matter

- Respirable particles PM 10 (smaller than 10  $\mu\text{m}$ )
- Respirable particles PM 2.5 (smaller than 2.5  $\mu\text{m}$ , reach the alveols)
- Particles are carrier of e.g. ROS generating organic compounds
- Metals (can catalyse reactions of harmless substances into noxes)

## Indoor pollutants

- Smoke from indoor combustion (toxic gases and particulate matter)
- Cigarette smoke (toxic gases and particulate matter)

Cigarette smoke is a good model for all three components



# Anti Pollution

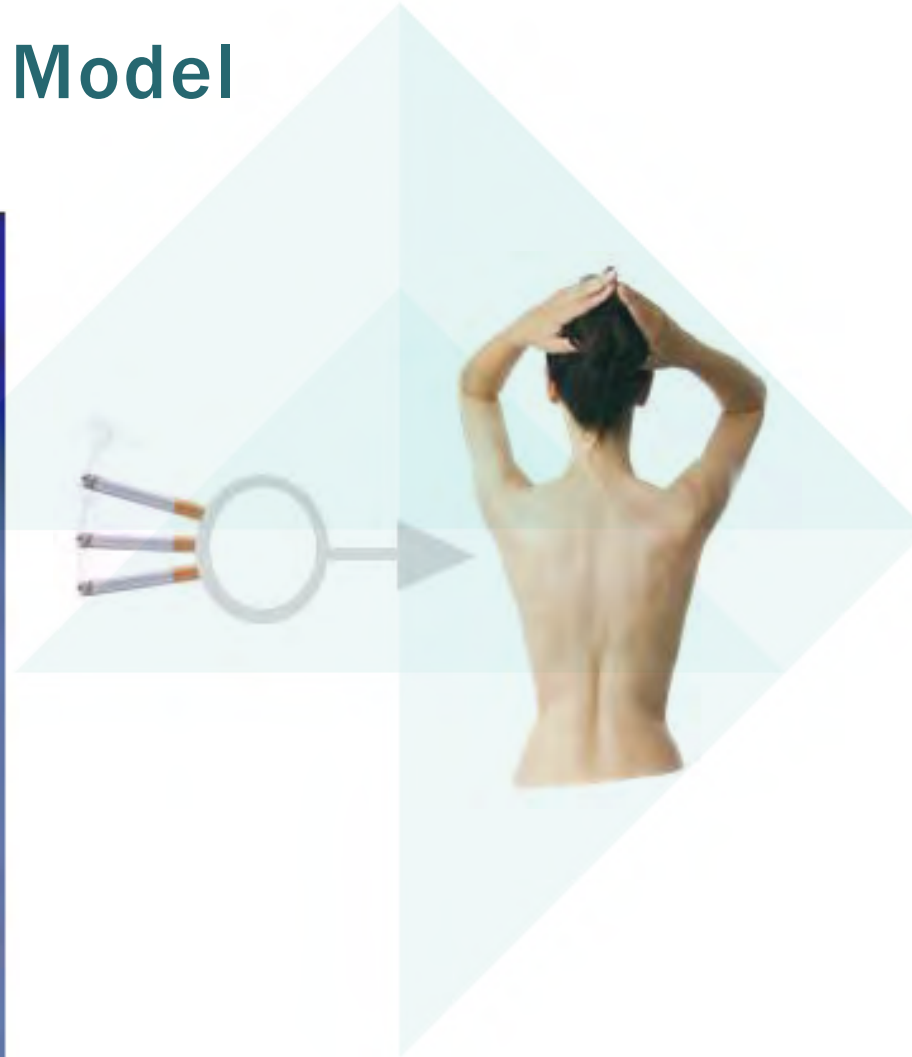
- Strategies of cosmetic products to protect skin from pollution:
  - Improve skin cleansing to thoroughly remove:
    - Reduce adherence of particulate matter (PM)
    - Wash off PM, even when the size is below 2.5  $\mu\text{m}$
  - Formation of a skin barrier by shielding :
    - Apply products that form a protective film
    - Keep toxic and ROS generating molecules away from skin
  - Application of antioxidants:
    - Replace consumed skin own antioxidants
    - Inactivate ROS molecules before they can react with the skin



# The Cigarette Smoke Model



Deposit of PM and tar on volar forearm, VIS and Fluorescence light



- Assessments are performed in vivo on the back or volar forearm
- Fresh cigarette smoke is used as the model pollutant
- Assessment of oxidized lipids from sebum and / or skin barrier



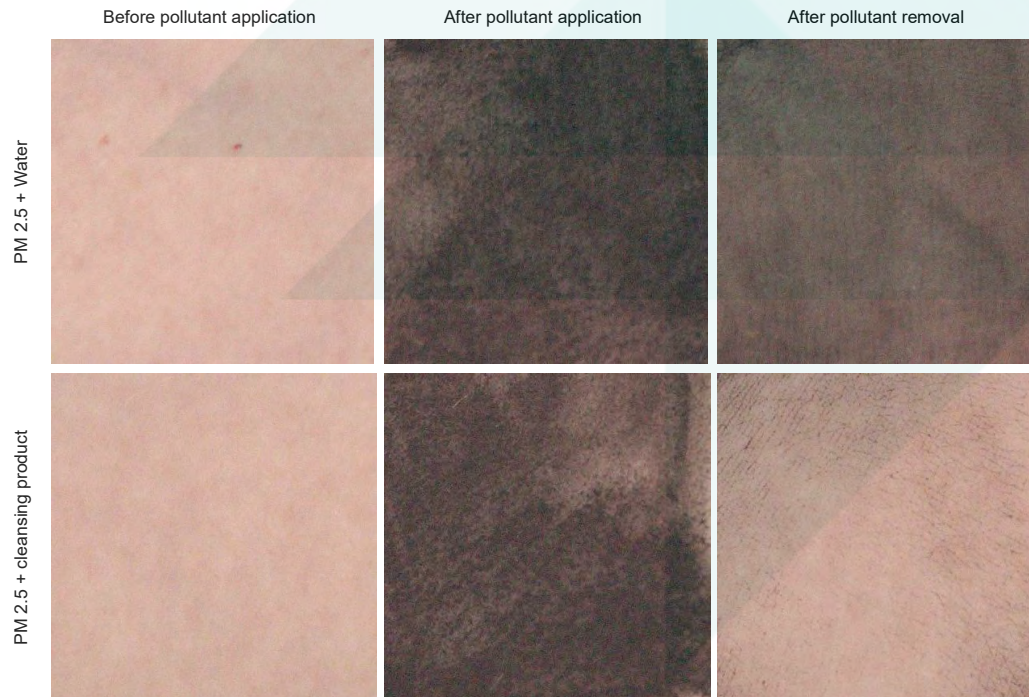
# Possible Test Designs

	Instant protection	Long term protection
Subjects	10	
Parameters	Quantification of Squalene Monohydroperoxide (SQQOH), Malondialdehyde (MDA) LC/MS or GC/MS determination by external lab 'Synelvia'	
Test area for barrier lipids	5 testing areas on the volar forearm	
Test area for sebum lipids	5 testing areas on the upper back	
Test products	3 cosmetic products, positive control (untreated area exposed to smoke), negative control (untreated area not exposed to smoke)	
Test procedure	Day 1: Product application, 15 min. waiting time, 15 min. exposure to pollution, sampling	Day 1, 2, 3, 4: Product application After 24 h, on day 5: 15 min. exposure to pollution, sampling



# Cleansing Claim

## PM 2.5 Carbon Particles: Effect of a Cosmetic Cleanser versus Water



# Skin Aging by Environmental Pollution?



## Claim Support for Anti-Pollution Cosmetics

The proderm Webinar on In Vivo Anti-Pollution Testing

Stephan Bielfeldt

**Skin Research & Technology**



ORIGINAL ARTICLE

**Anti-pollution effects of two antioxidants and a chelator—Ex vivo electron spin resonance and in vivo cigarette smoke model assessments in human skin**

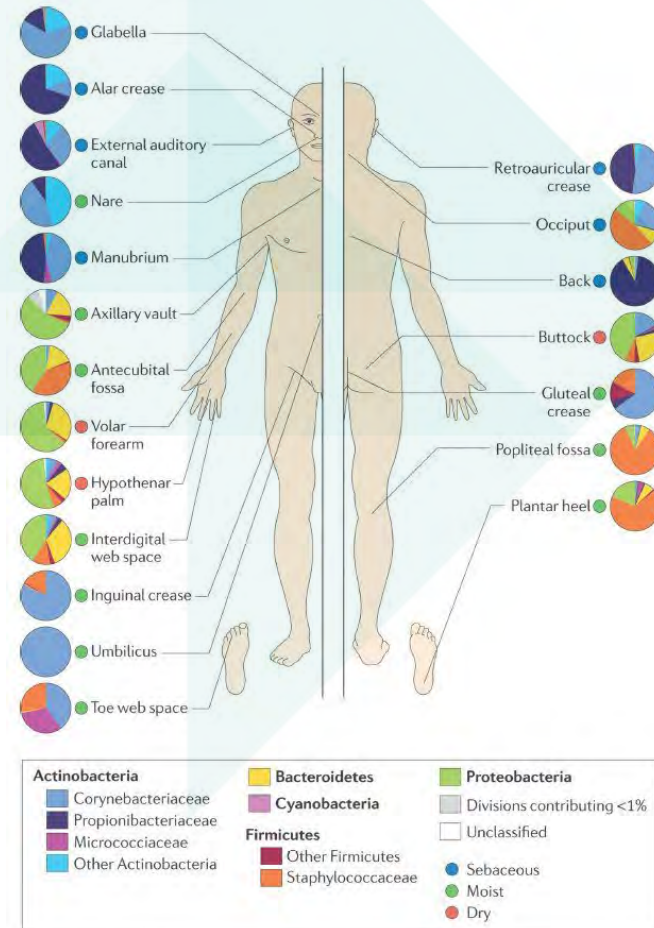
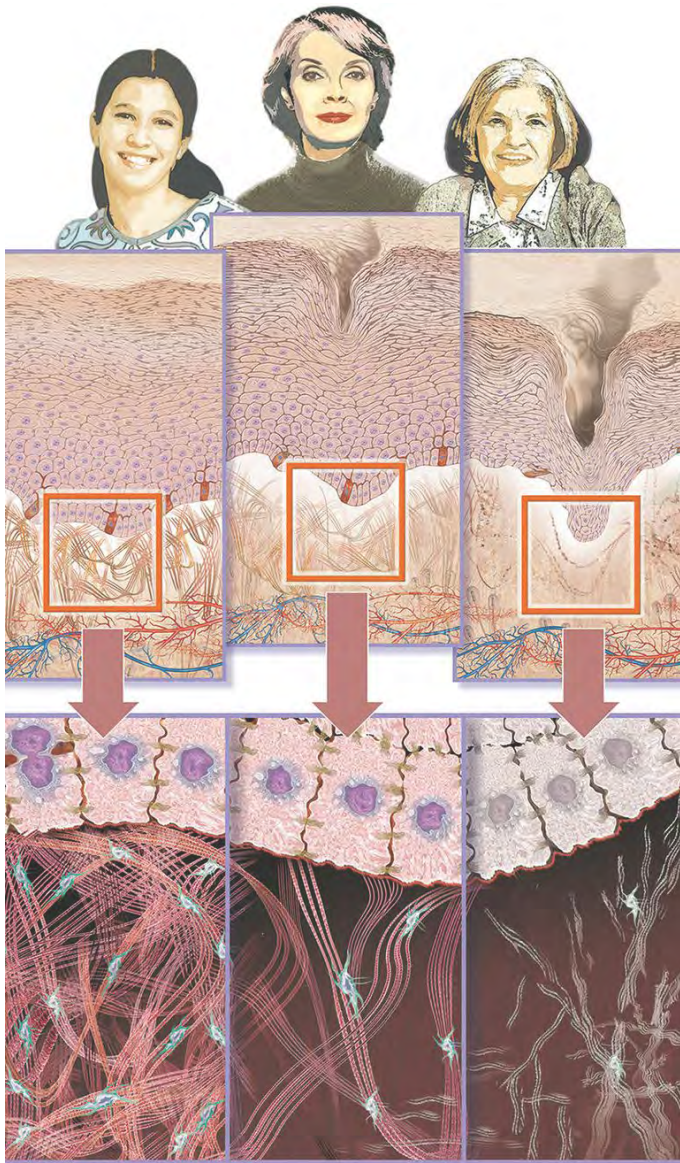
Stephan Bielfeldt ✉, Katinka Jung, Sabrina Laing, Alain Moga, Klaus-Peter Wilhelm

First published: 10 June 2021 | <https://doi.org/10.1111/srt.13068>



# Protective layers in skin aging

## Microbiome







# Study Design Microbiome



- Sampling: swabbing of test area, and soaking in special buffer, storing of samples (-80°C)
- Microbial Analysis:
  1. analysis by the 16S phylogenetic profiling method based on sequencing the 16s rRNA gene.
  2. Whole Genome Shotgun – Metagenomic Sequencing “Shotgun” refers to the process in which total DNA is fragmented in a random manner before sequencing
  3. Shallow Shotgun metagenome sequencing (SSMS)



# proderm Quality Seal for Microbiome

- 30 subjects
- Product application over 4 weeks after a washout phase
- Analysis of microbiome at beginning and end
- Combined with further investigations:
  - Dermatological evaluation of skin status
  - Check of skin pH and skin barrier function
- „Maintains microbiome“ or „microbiome-friendly“





## Collection of tapes for analysis of AMPs (Antimicrobial peptides)

- Tape Sampling for AMP Analysis
- Tapes will be taken to analyze the following concentrations of AMPs in stratum corneum by ELISA kits such as hBD-2 (Human Beta Defensin 2), HBD-3, RNase7, Psoriasin

*Maja-Lisa Clausen et al. Measurements of AMPs in stratum corneum of atopic dermatitis and healthy skin—tape stripping technique 2018 Scientific Reports*



# Anti-age/Anti-wrinkle Claims

## Established acceptable claims:

- Helps prevent signs of aging
- Refresh your looks
- regenerate your skin's appearance
- renew the look of your skin
- Reduces fine lines slows the signs of premature aging
- Reduces the signs of aging
- Minimizes fine lines
- feel (look) younger (youthful)
- helps prevent (reduce, slow) the signs (appearance) of ageing (age lines, premature ageing)
- moisturise ageing skin
- smooths wrinkles
- Anti-wrinkle cream/anti-wrinkle moisturizer

### 3.3.21. Anti-wrinkle products

160. **Question:** Are anti-wrinkle products cosmetic products?

161. **Answer:** Yes, in principle anti-wrinkle products can be cosmetic products. This is confirmed by the listing of anti-wrinkle products in Annex I to Directive 76/768/EEC and recital 7 of the Cosmetics Regulation.

162. However, some products presented as anti-wrinkle may significantly restore, correct or modify physiological functions by exerting a pharmacological, immunological or metabolic action. In that case, they would not qualify as cosmetics.

163. A decision on the qualification of the products has to be made by the national competent authorities on a case-by-case basis, and taking into account all the relevant elements, such as, for example, the presentation of the product, any promotional literature, the composition, the product's specific pharmacological, immunological or metabolic properties, the mode of application under normal and reasonably foreseeable conditions of use, the frequency of application, the application site, the degree of penetration and the risk which its use may entail.

164. In order to achieve the anti-wrinkle effect, several substances are typically used and have different modes of action.

165. Some substances - such as tretinoin<sup>64</sup> (all-trans retinoic acid), gerotone<sup>65</sup> (spermine), phenol<sup>66</sup> and progesterone<sup>67</sup> are banned when used in cosmetic products. In this case, if anti-wrinkle products containing these ingredients are marketed as cosmetics, they are illegal cosmetics.

# Questions

