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Collagens and skin aging – background and test methods
.

Marianne Brandt, Division Manager Cosmetics, June 08,2023

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Contents

- What is collagen?
- Collagen and skin aging
- Environmental influences
- Product types
- Methods

What is collagen?

- Protein in human dermis
- Makes up 75% of the dry weight of dermis / 90% of total protein content of dermis
- Collagen and elastin embedded in gel-like extracellular matrix of the dermis

- Young skin = thin reticulated collagen fibers, larger and less fragmented collagen network
- Old skin = more coarse and huddled collagen fibers, less organized collagen network

- Collagen molecules first synthesized as procollagen; they develop in extracellular matrix to collagen fibers
- Concentration of procollagen propeptides reflect the local collagen synthesis, can be measured in body fluids

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Aging on the epidermal level - Epidermis

llustration: © Kleinhans RED,
source: www.skin-care-forum.basf.com.
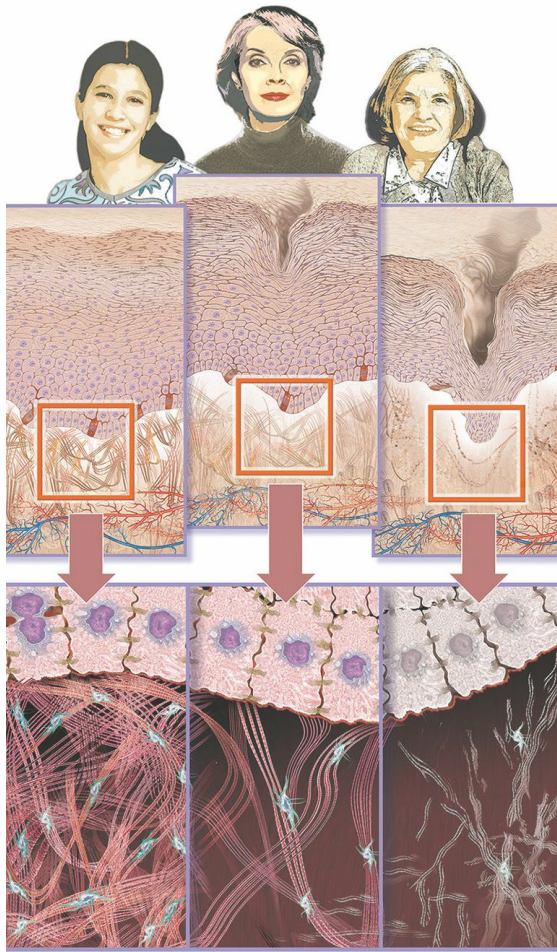
- Lipid content decreased (SC)
- Flattening of dermal-epidermal junction
- Number of enzymatically active melanocytes decreases by 8% to 20% per decade
- Number of Langerhan's cells decreases
- Capacity for re-epitheliazation diminishes

- Skin hydration ↓
- Sebum secretion ↓
- Elasticity ↓
- Skin surface pH ↑
- Protection and permeability barrier ↓
- Pigmentation ↑
- Decreased lipid content and altered lipid organization
- Desquamation & proliferation ↓
- Healing and immune function ↓

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Aging on the dermal level - Dermis
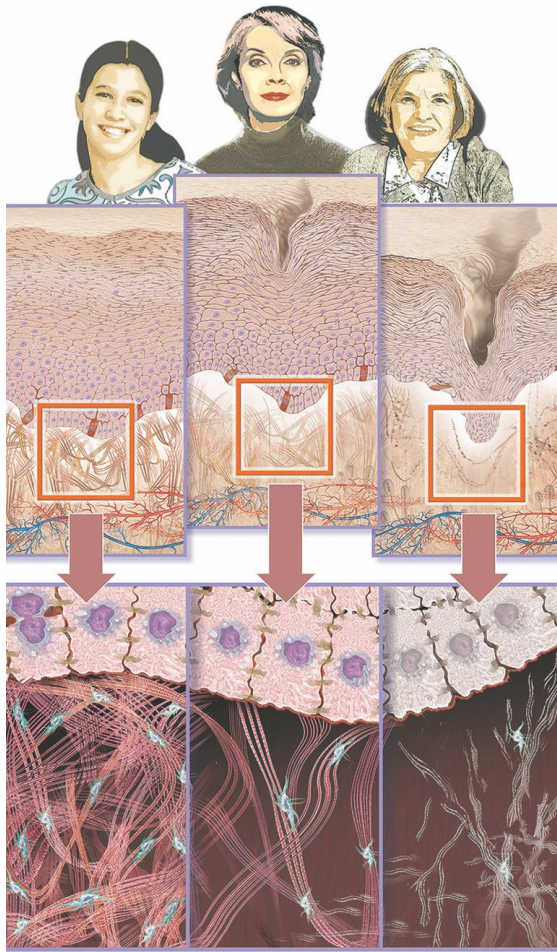
llustration: © Kleinhans RED,
source: www.skin-care-forum.basf.com.

- 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 ↑

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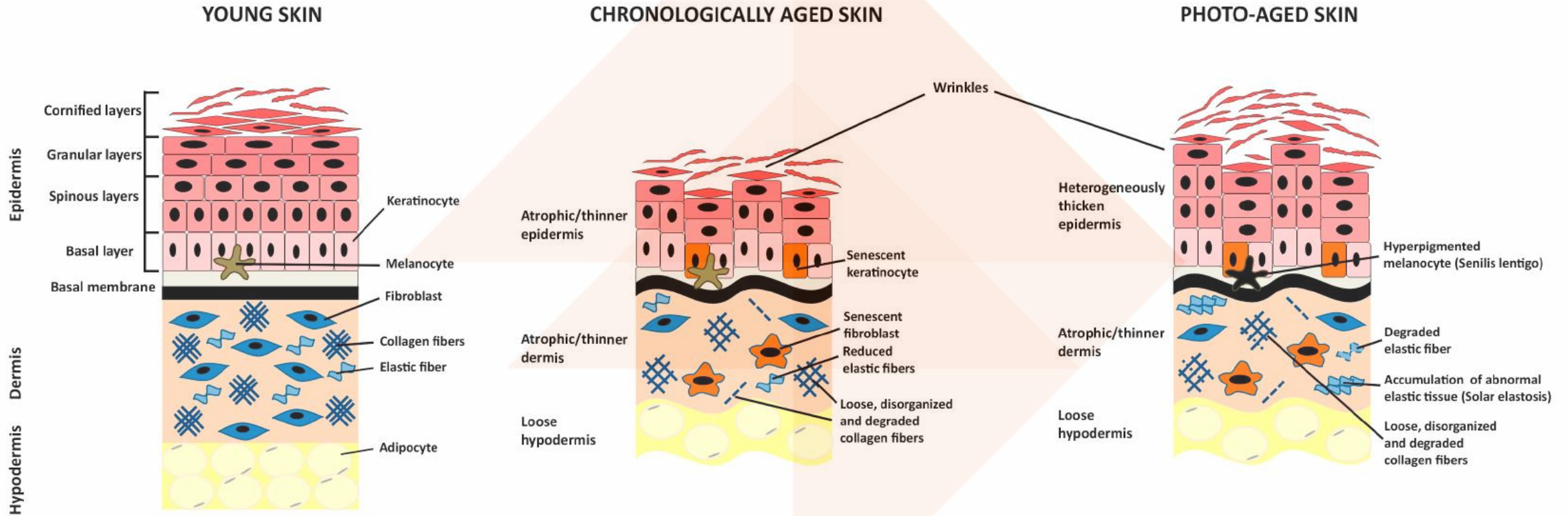
Orioli, D., & Dellambra, E. (2018). Epigenetic regulation of skin cells in natural aging and premature aging diseases. *Cells*, *7*(12), 268.

Environmental Factors - The Skin Aging Exposome: Causes are Manifold
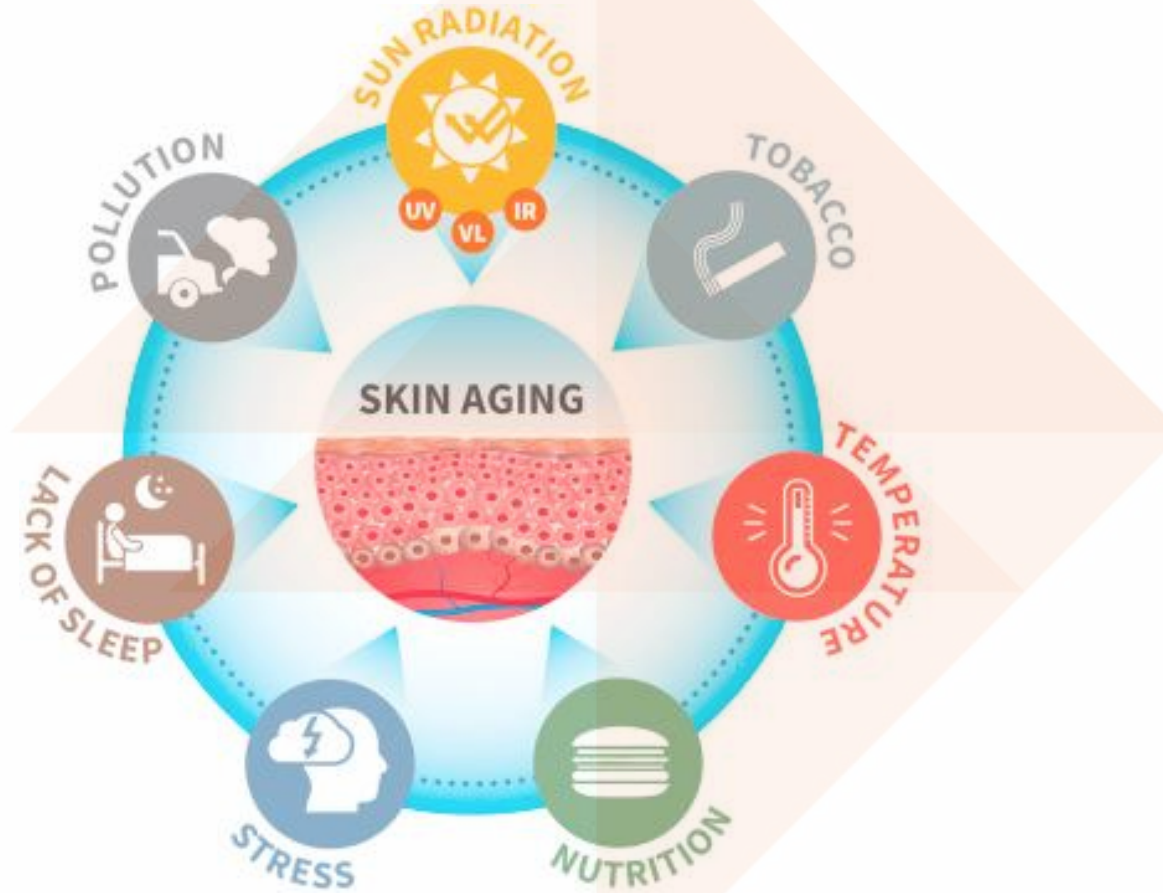
Krutmann, J., Bouloc, A., Sore, G., Bernard, B. A., & Passeron, T. (2017).
The skin aging exposome. Journal of dermatological science, 85(3), 152-161.

Which products look at aging with emphasis on collagen?

- Topically applied creams – cosmetics
- Collagen-containing drinks or pills - food supplements
- Aesthetic treatments, fillers – medical devices/ medicinal products

- Careful consideration of how claims are made to fulfil the respective regulations!
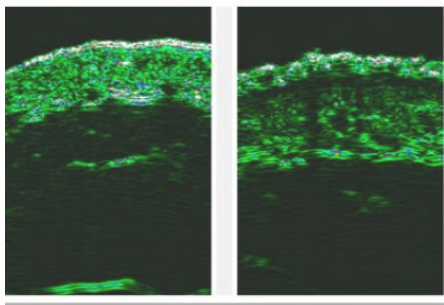
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How can we measure effects of these products?

- Measurement of skin elasticity, firmness
- Correlates with age

- Possible claims:
  - Improves Firmness
  - Improves Elasticity
  - Tonicity and/or Suppleness
  - Skin feels/appears lifted

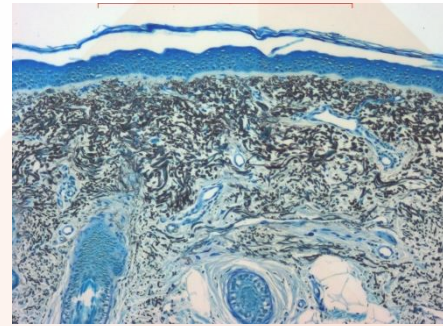Cutometer® MPA 580, Courage+Khazaka
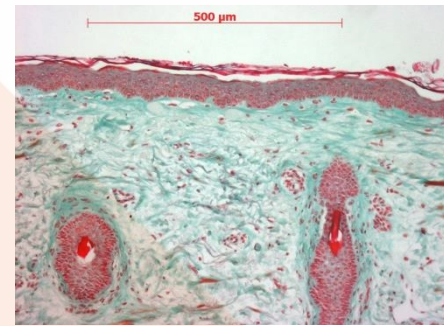Electronic GmbH, Köln, Germany
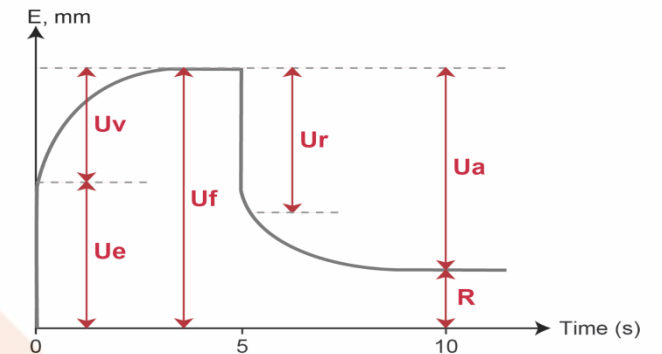
How do we evaluate effects on collagen?
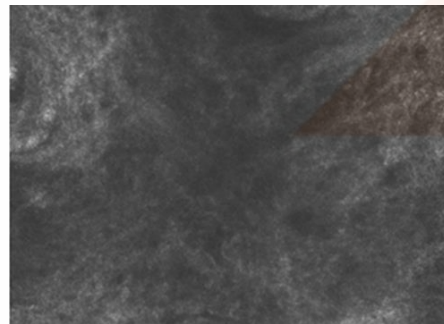

22Mhz-Ultrasound

Vivosight (OCT)

Orcein staining from biopsies (black elastic fibers)

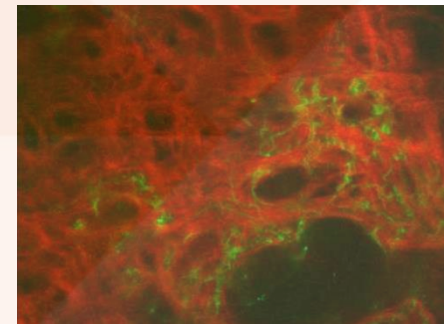Masson-Goldner staining from biopsies (green collagen fibers)

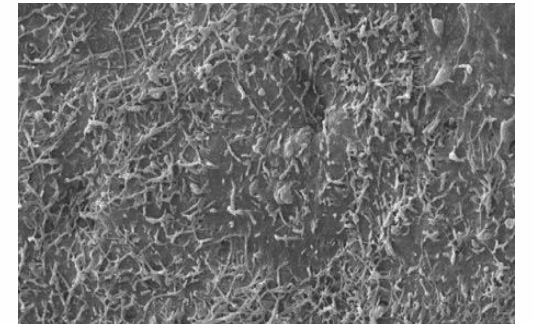
Confocal Microscopy (Vivascope)
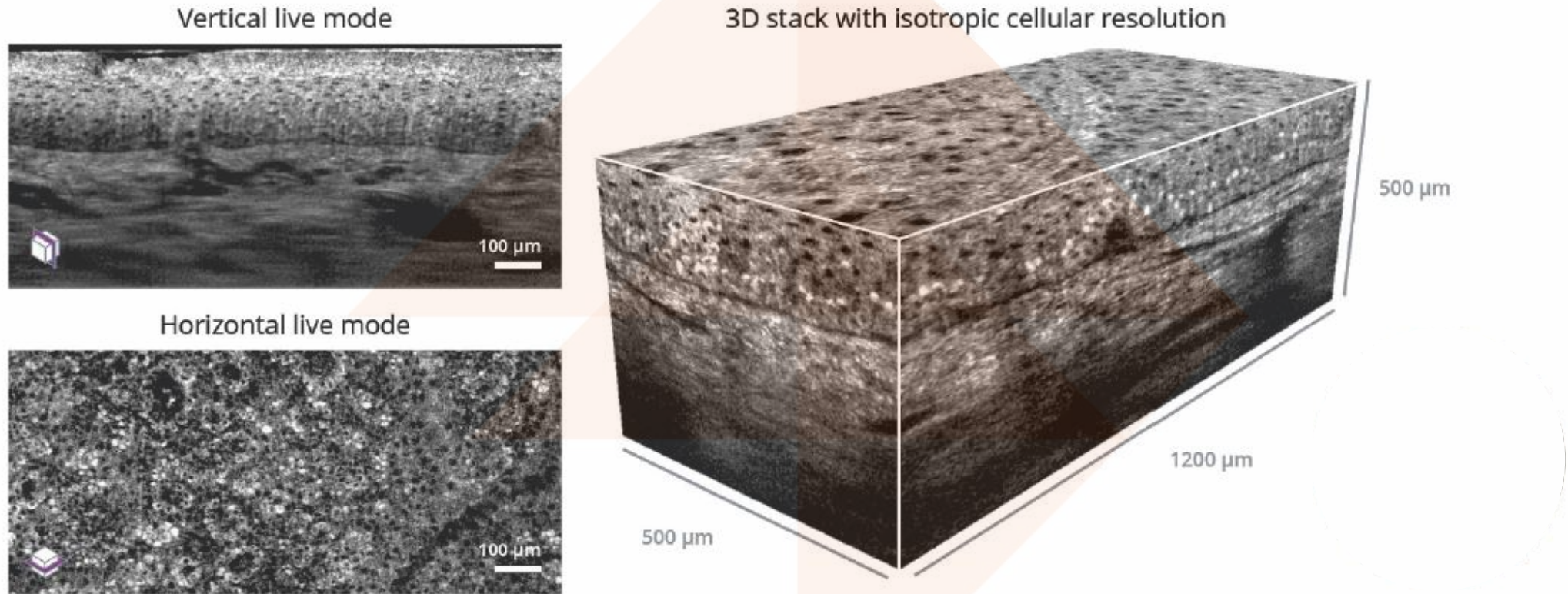

Collagen image from Vivascope

Multiphoton Tomograph

Autofluorescence of elastin and collagen crosslinks (green) and collagen SHG-signal (red)
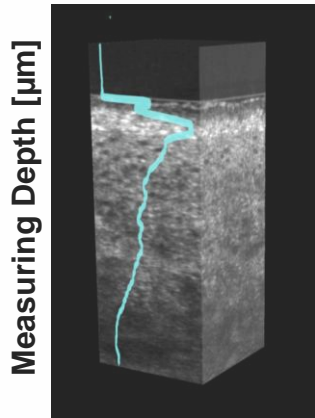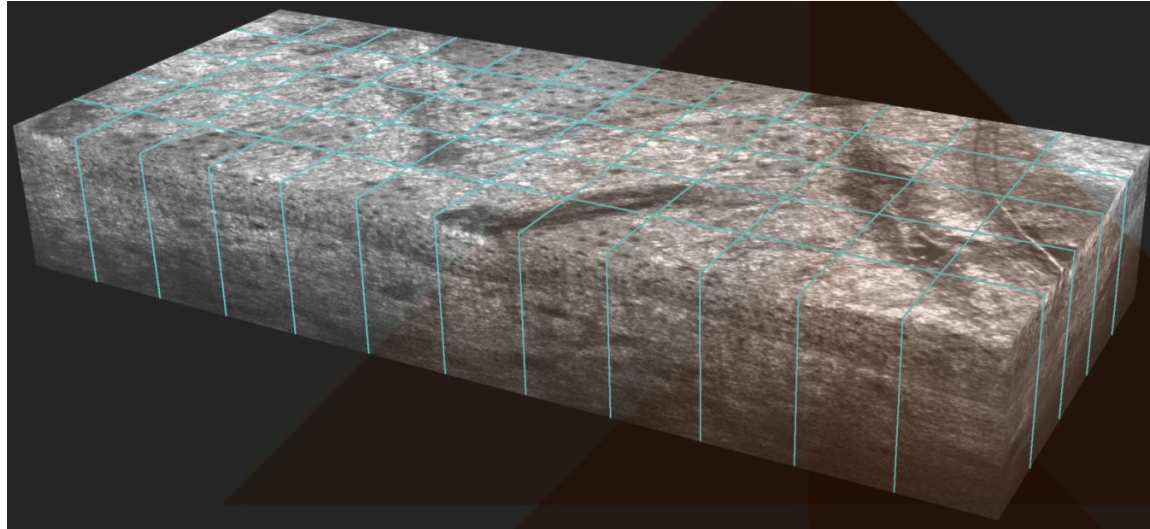

TEM evaluation of suction blister roofs

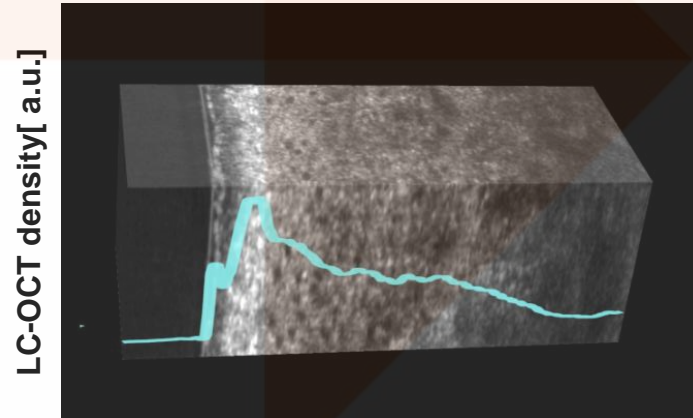SGS proderm

Are there other ways to evaluate effects on collagen? LC-OCT

Vertical live mode

Horizontal live mode

3D stack with isotropic cellular resolution

100 µm

100 µm

500 µm

1200 µm

500 µm

LC-OCT – Imaging of epidermis, papillary and reticular dermis down to ~ 400 µm; resolution (x, y, z) ~ 1 µm
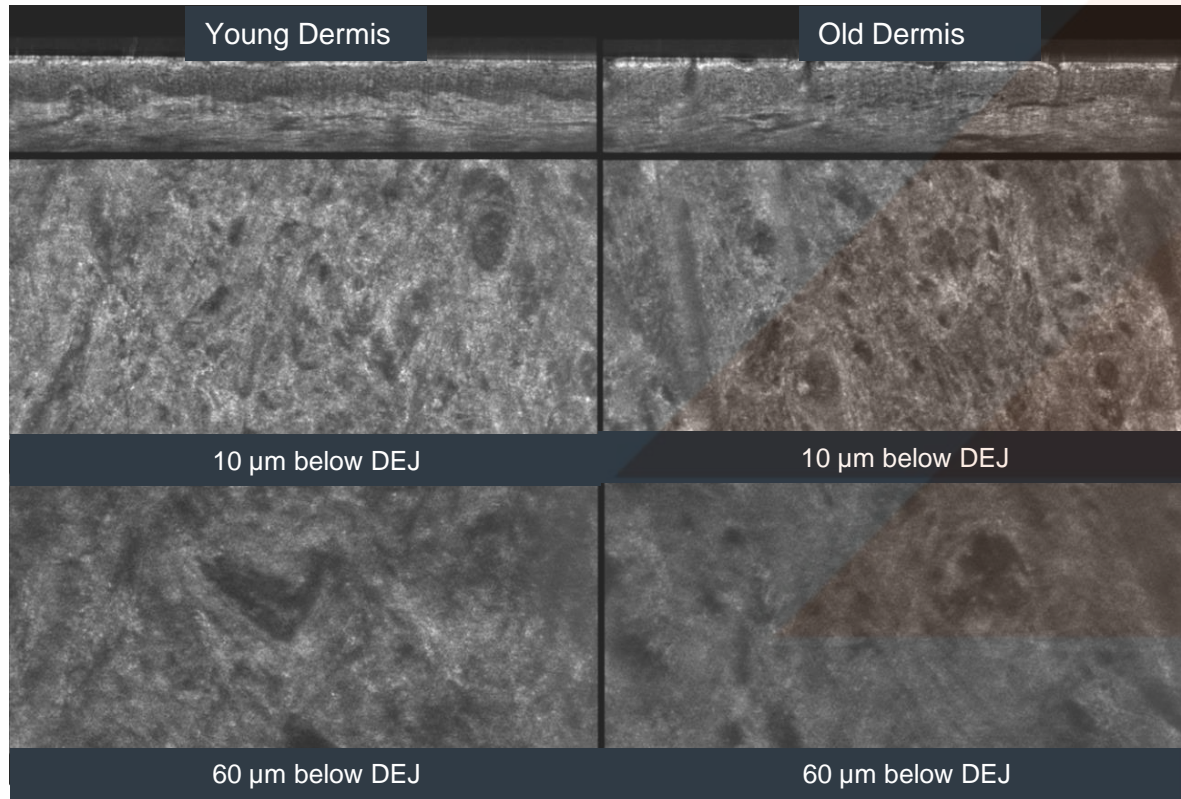
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LC-OCT – Optical Attenuation
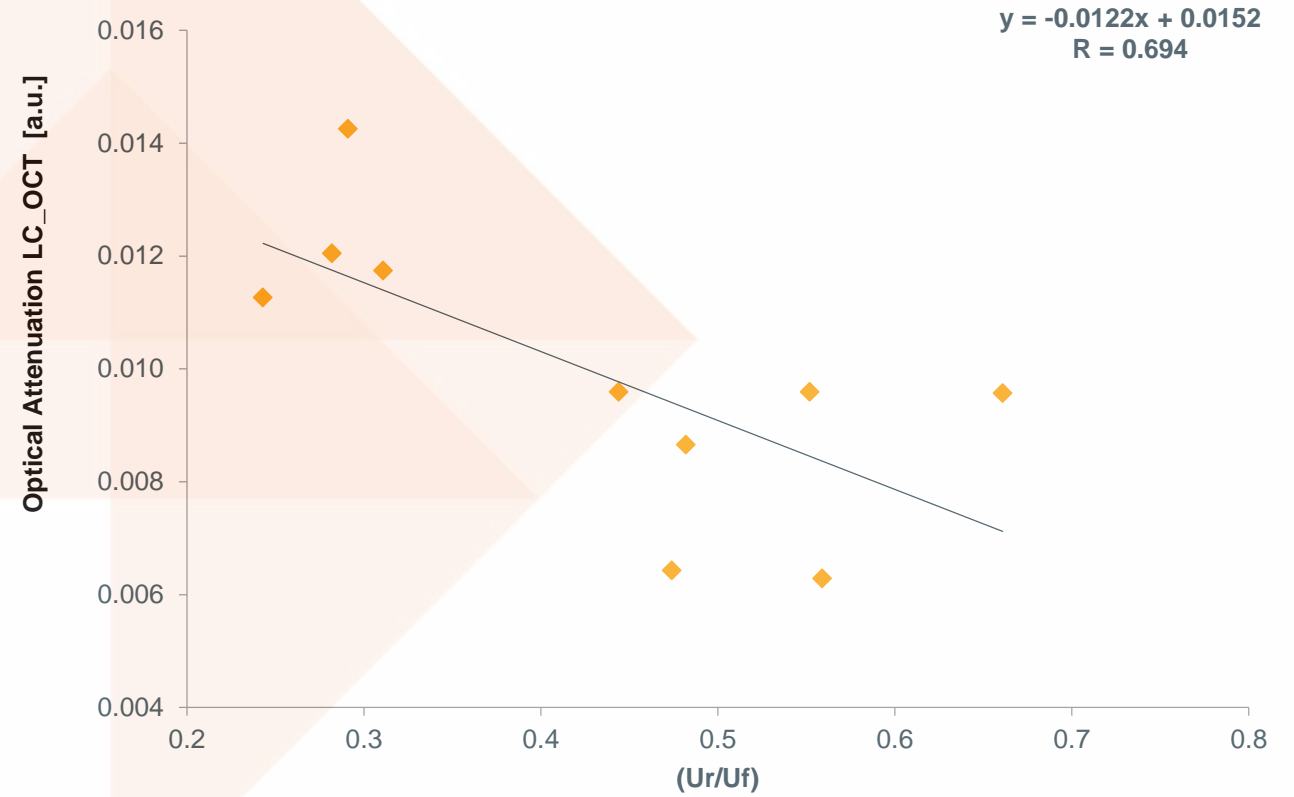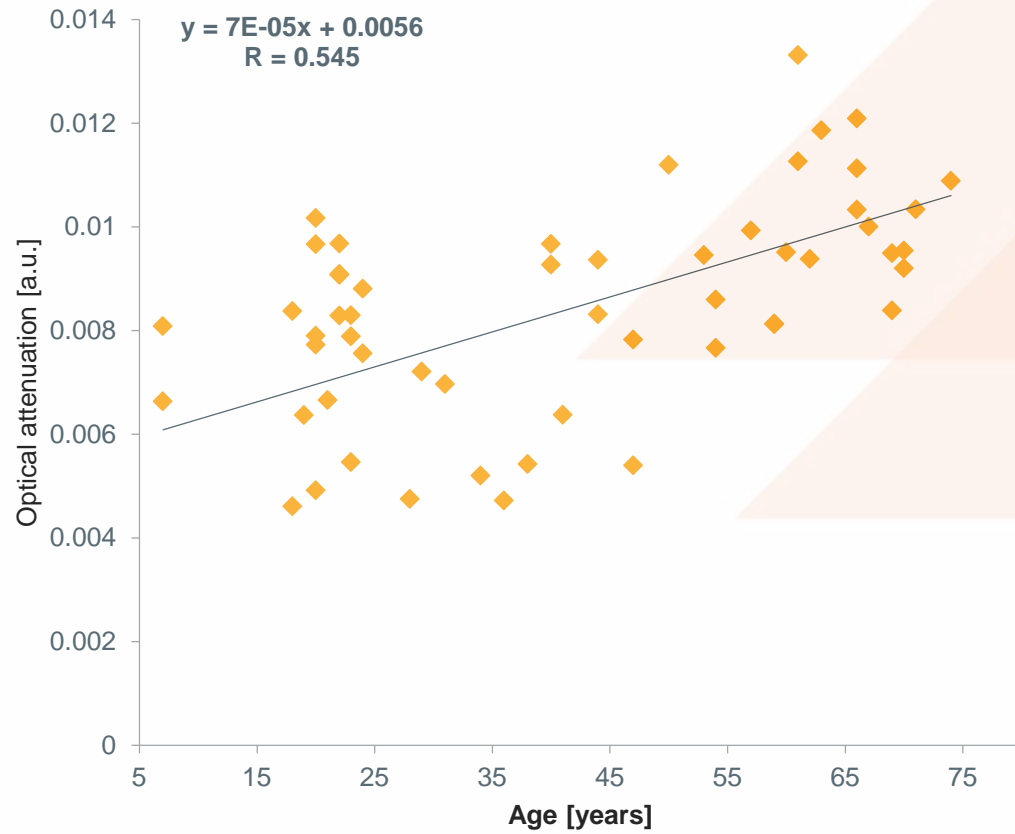
LC-OCT – Optical Attenuation
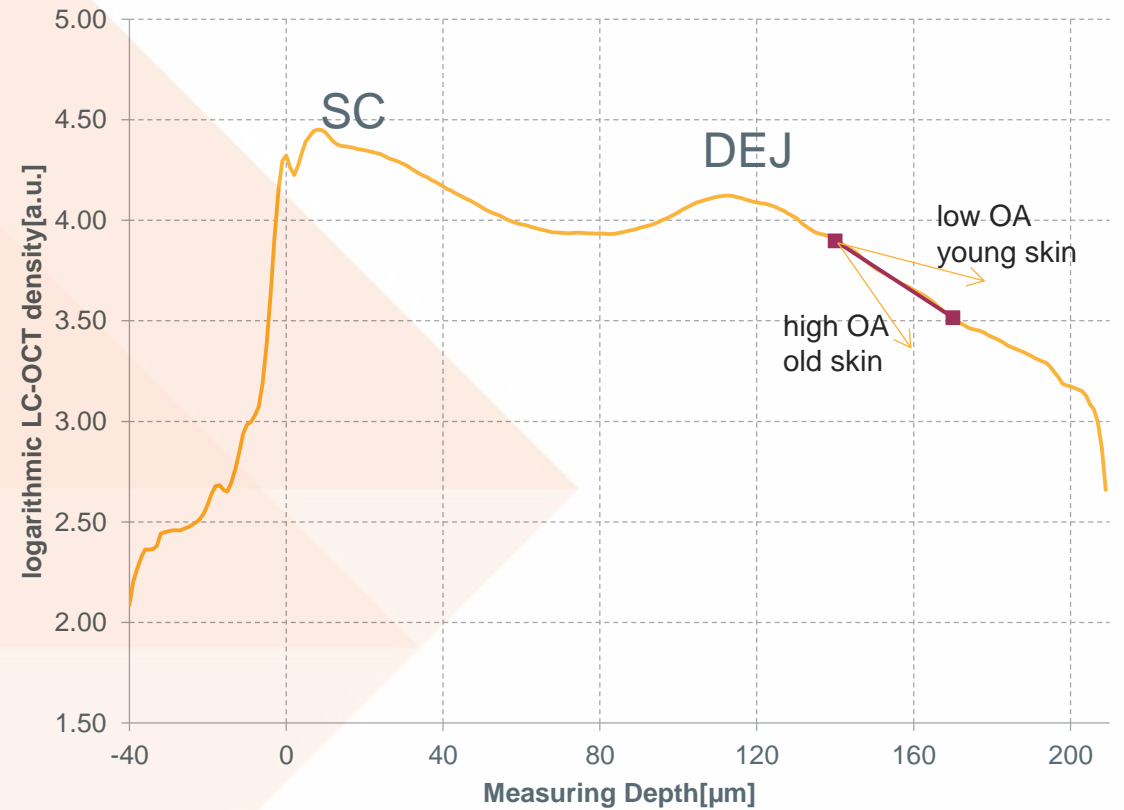
Optical Attenuation: The negative slope of the LC-OCT signal in the upper dermis
Young skin => high fibre reflection => low Optical Attenuation
Aged skin => low fibre reflection   => high Optical Attenuation

LC-OCT – Correlation with age and Cutometer results

$y = 7E-05x + 0.0056$
$R = 0.545$

$y = -0.0122x + 0.0152$
$R = 0.694$

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LC-OCT – Optical Attenuation

- Determined in a depth of approximately 150 to 200 μm

- A layer where strong collagen bundles are present which weaken with aging

- The more reflective material appears, the lower the optical attenuation

- Association with improvement of collagen structure
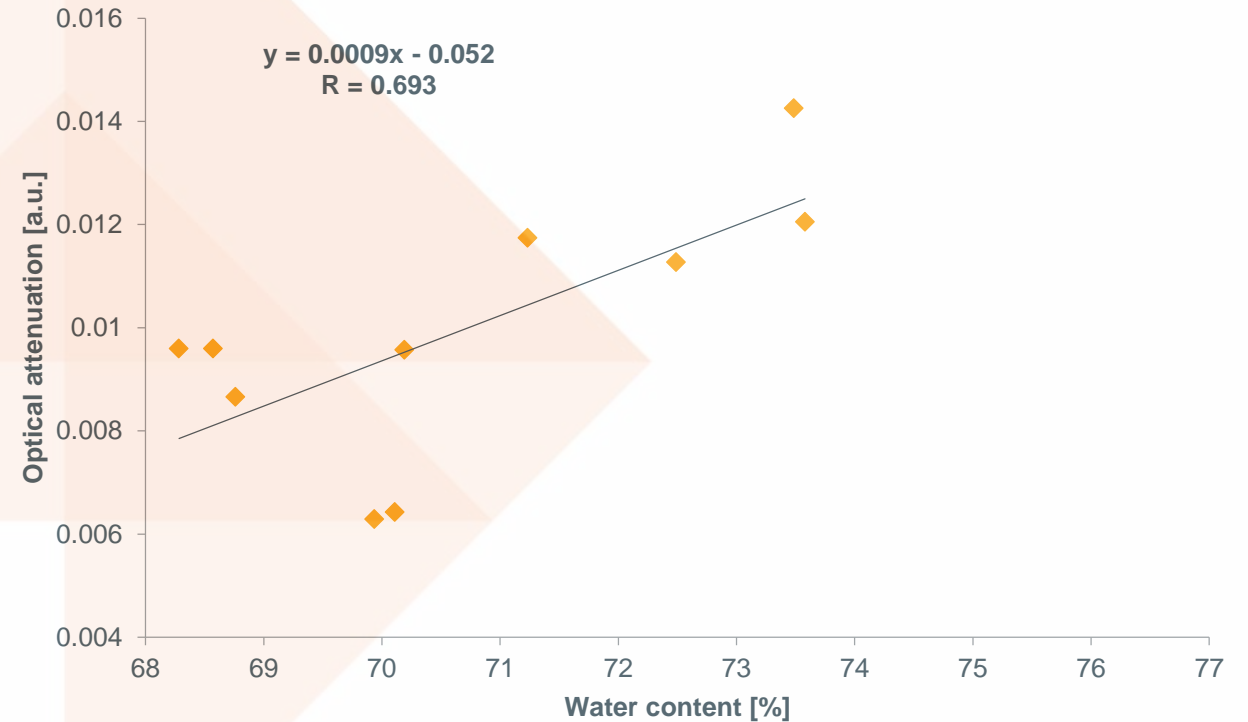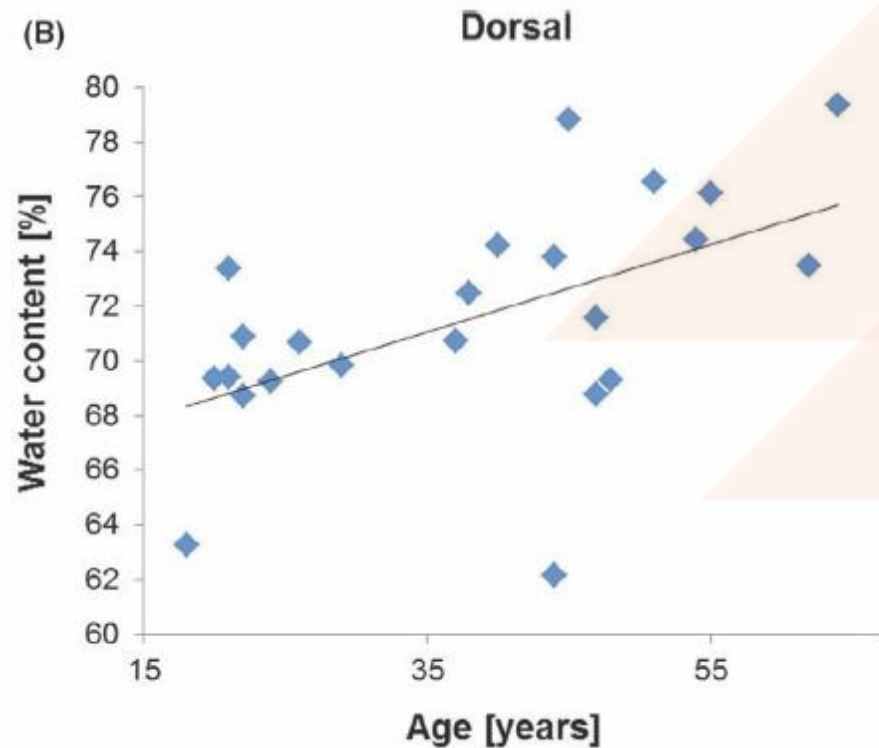
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Are there other ways to evaluate effects on collagen? Raman
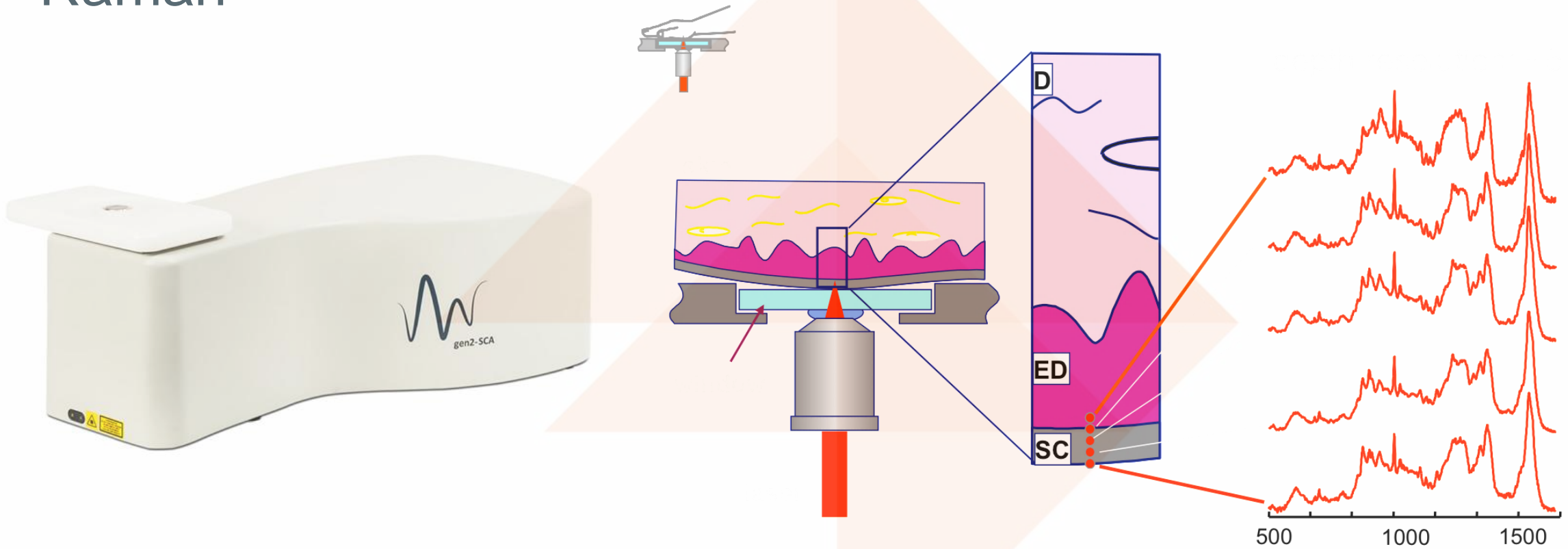
Raman – Measuring molecules in upper layers of skin down to dermis, water content in skin

Raman – Correlation with age and LC-OCT results

(B) Dorsal

Measurement at a depth of 130 to 150 µm (upper reticular dermis)

$y = 0.0009x - 0.052$
$R = 0.693$

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Are there other ways to evaluate effects on collagen? Suction blister method to analyse procollagen

In vivo treatment

▼

Non-invasive ex vivo sampling
Complete viable epidermis with intact basal cell layer (blister roof) plus interstitial fluid within the blister

▼

In vitro analysis and diagnosis
(e.g. Biochemistry, Cell Biology, Immunology, Molecular Biology, Histology, etc.):
ELISA assay (Enzyme-Linked Immunosorbent Assay) for synthesis of procollagen in the interstitial fluid of suction blister biopsies

Suction blister induction by atmospheric subpressure

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How does it look like in studies?

- Study on at least 30 subjects per product

- 4 to 6 months duration

- Comparison versus untreated or placebo

- Collagen- specific parameters: LC-OCT and/ or suction blister with analysis

- Combined with

  - skin elasticity

  - skin hydration

  - 3D profilometry

  - questionnaire on product acceptance and performance

  - questionnaire on well-being

  - clinical photography

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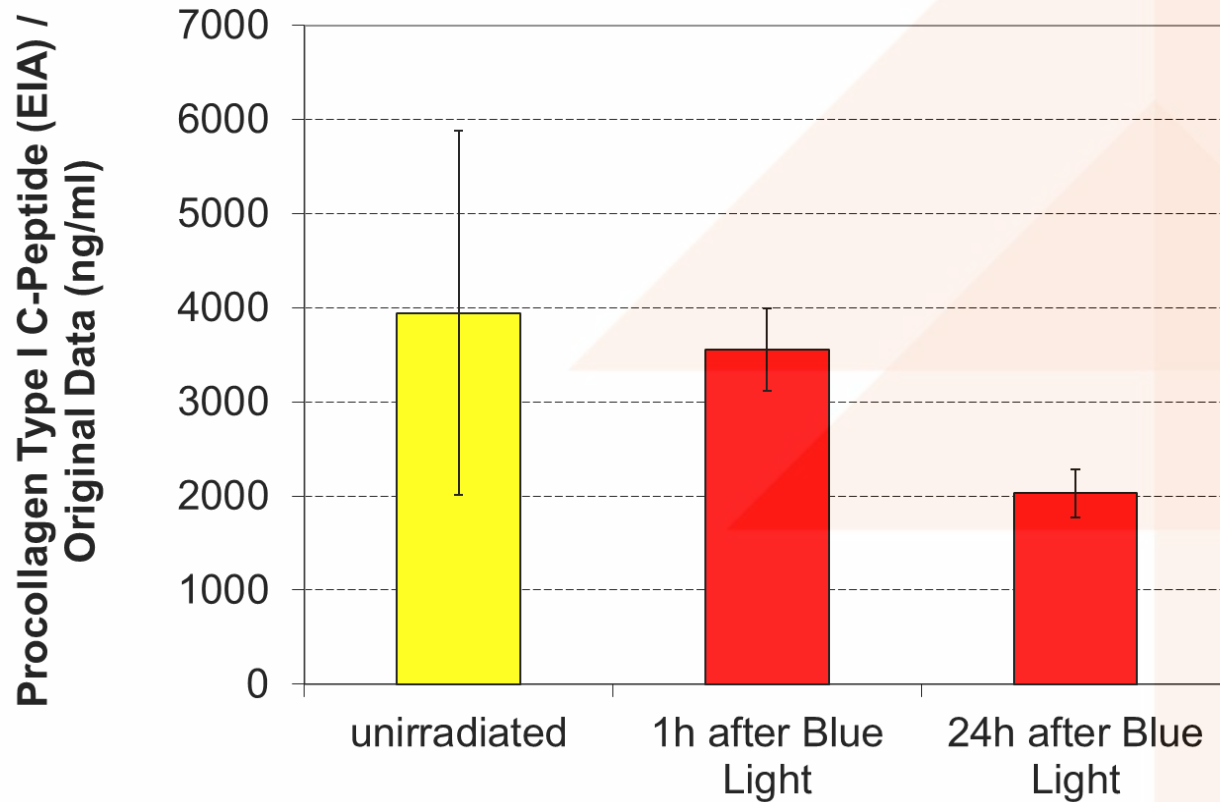# How does it look like in studies?

Optical Attenuation
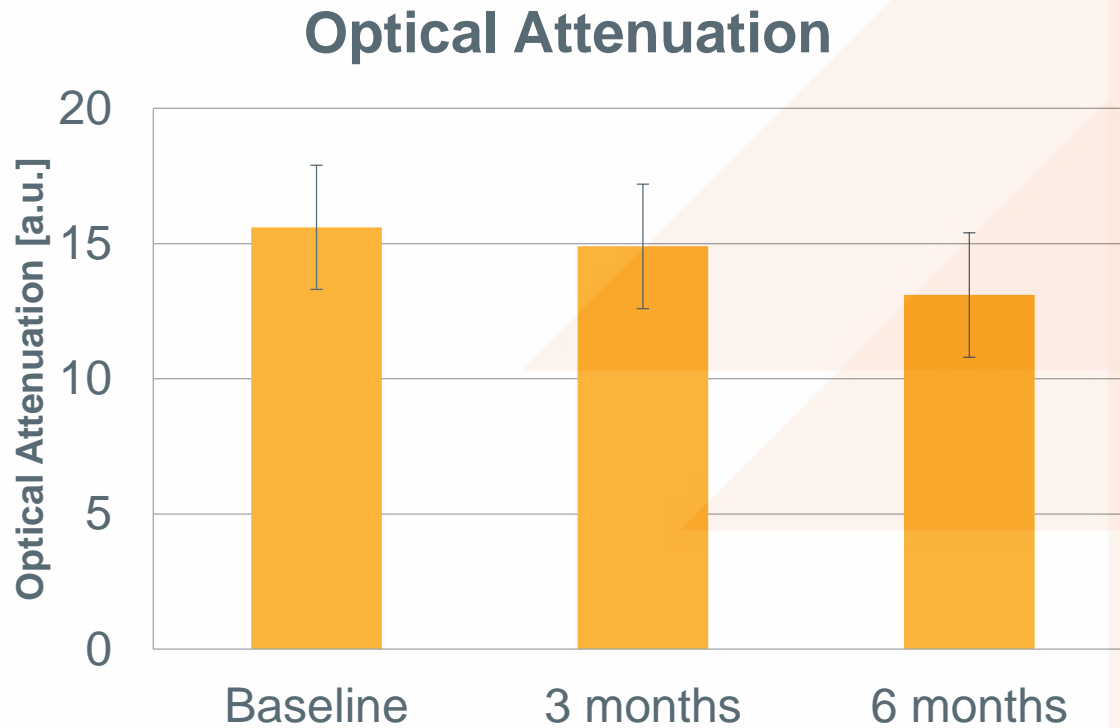
- Decrease in OA = more reflection = improved collagen structure
- Typically in line with increased SC thickness
- Increased epidermis thickness

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How does it look like in studies?
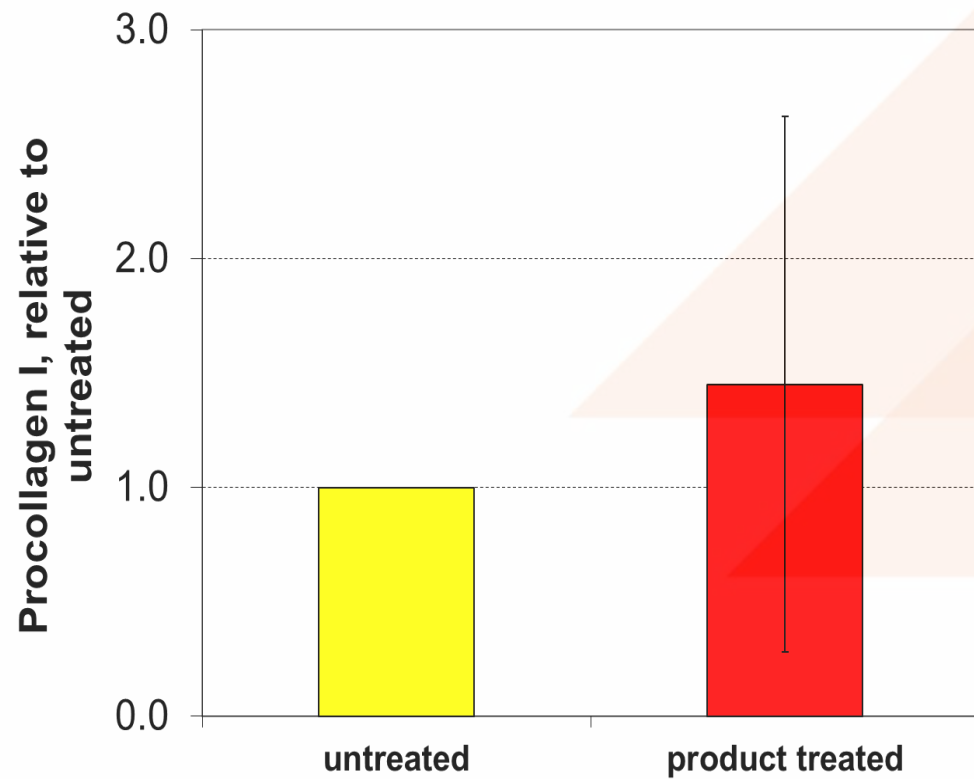
- Increase in procollagen **I** as benefit to improve collagen structures

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How does it look like in studies?

- Depletion of procollagen I due to blue light irradiation

Summary – Take home messages

- Raman water measurement in the dermis: efficacy of anti-aging and anti-photoaging products, with an indirect link to collagen via water content assessment

- LC-OCT and optical attenuation: non-invasive and in vivo measurements of age-dependent depletion of collagen fiber network in dermis

- ELISA/ suction blister: a well-known assessment of procollagen I, a pre-stage to collagen, with a direct statement to the current collagen status of the skin

- In combination, the methods give the possibility to visualize and quantify different aspects of measuring collagen in the skin to substantiate claims for products' efficacy

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Questions?

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